

Dead-end for British research

By parsimony, indolence and indifference, the British Government is killing off imaginative research. It must act quickly if it cares to retrieve the situation. But it may already be too late.

NOT so long ago, British governments were forever congratulating themselves that British scientists had what seemed to be an inside track in the competition for Nobel Prizes. Per head of population and by most other yardsticks, the roll of honour has been so ample as to seem endless, even effortless. For much of the past half-century, British scientists, while acknowledging that the statistic is spurious, have enjoyed the kudos that distinction brings and have made a virtue of what seems to have been the perpetual necessity of sustaining research on a shoestring: "if we can achieve so much with only string and sealing-wax, what might we not accomplish with the proper tools?"

Since 1945, both British scientists and British governments have been dismayed that this apparently innate flair has not engendered national prosperity. The most common resolution of that conundrum has been in proud disdain: who would ask that Beethoven should have been skilled at selling sheet music? But, fitfully, British scientists and their governments have collaborated on ill-prepared technical projects intended as means of using the same flair to capture a commanding lead over more methodical industrial competitors. The list of disasters following from these decisions is also endless — the Comet aircraft, ZETA (the peaceful thermonuclear device that proved to be a roundabout way of heating electrons), Bluestreak (the homespun ballistic missile that never left the ground), half of Concorde, the High-Speed Passenger Train, the information technology minister's plan of 1982 to "cable Britain".

To the extent that such projects have sprung from the tension between smug security in scientific achievement and frustration at the lack of material benefit therefrom, the list of spectacular disasters should soon end. The British Government has done much to induce a sense of realism among those who would turn science into prosperity; they know now how skilled and well-equipped is the opposition. But the tension will melt away because the research enterprise is on its last legs. The goose that might have laid those golden eggs will not be around much longer.

It will be some time before British scientists are again prominent on the Nobel lists. Dr César Milstein, the Cambridge Argentinian, will no doubt at some stage be recognized. Justice may also yet be done to Sir Fred Hoyle. Otherwise, there is nothing in the recent record of British research laboratories that lifts the spirit and stretches the imagination as much as the dozen or so outstanding discoveries each year at laboratories elsewhere. The British scientific community will comfort itself that it is doing the best it can with inadequate resources. British governments will learn to parrot that Nobel statistics are spurious, and that it matters more that British industry should be able to turn a nearly honest penny, devaluing now by only 5 per cent a year.

It is unkind to kick even governments when they are down, as the Thatcher Government has been these past few weeks. But they must be helped to see the errors in their ways, especially when their own perspective is distorting. The British Government has lived up to Mrs Thatcher's promise, four years ago, that the science budget, government support for basic science, would be "protected". Why should this journal now be saying that the stuffing has gone out of the scientific enterprise? And that the government is to blame?

The charge requires proof, for the British scientific community is not above special pleading on its own behalf. Many taxpayers

will know of particle accelerators advocated as essential means of keeping the scientific enterprise in good health but then quickly abandoned. The four research councils that are the conduits for public money for basic research find themselves supporting institutions founded in happier times that now seem extravagances, even distractions. The Science and Engineering Council's Neutron Spallation Source in Oxfordshire is one such luxury, but there are many others. And the now-common complaint by university researchers that adventurous work is impossible for lack of funds is superficially an amplified version of the perennial excuse of the unsuccessful.

Struggle

The novelty that has now arisen is not the shortage of funds for research, acute though that may be, but the way basic research has become a hand-to-mouth struggle. At the highest level, the research councils are forever having to readjust their plans to new estimates on how little there will be to spend. (The Medical Research Council last week was faced with saving £700,000 next year and £2 million in each of the two succeeding financial years.) Even in well-equipped laboratories, people who have scraped through this year by running down their stock of consumable materials have no idea what will happen next. Able researchers are doubling as their own technicians. The British Government's palliative for 1983, the scheme for appointing younger academics to university posts (called "new blood" lectureships), has not been matched with the wherewithal to allow these talented people to prosecute effective programmes of research. The use of central facilities, in Britain or overseas, depends on success in the fierce competition for soft money. Customary British postwar envy of the United States is now matched by the knowledge that colleagues and competitors in France and West Germany enjoy a greater sense of security. Even in particle physics, the ground that Rutherford showed two generations of physicists how to till, the future is now clouded: characteristically, nothing has been decided, but the Kendrew committee may recommend next year that Britain should pull out of the European collaboration which it helped to found. Is it any wonder that bright young people are being driven from research?

When *Nature* advertised earlier this year an editorial post, nearly half of the 600 qualified scientists who replied had embarked on a career in research. Many were people with their first postdoctoral fellowship behind them. Their common reason for pulling out was their sense of the insecurity of a position in research. In an occupation in which dedication does not necessarily win glittering prizes, it seems doubly cruel that even the opportunity to work hard is on a short-term lease. No doubt there is some way in which the British national interest will be served by the diversion of this talent to occupations outside laboratories, but the collapse of morale throughout the research enterprise is a high price to pay.

The means by which this sense of insecurity has been engendered have been catalogued *ad nauseam* over the past several months. The science budget (now £500 million a year in round numbers) has indeed been held constant, but the rats have been allowed at it. Other government departments than the Department of Education and Science have reduced the scale of their research commissions, the demand for research funds from



an increased number of trained people has grown, the cost of research equipment has grown faster than inflation — and the competition has intensified. The most serious source of the financial pressure on research is the collapse of the convention that universities will meet the overhead cost of academic research out of the budgets provided by the University Grants Committee. Since the general budget reduction for the universities decreed at the end of 1980, the assumption that university teachers will be competing equally for research grants from “well-found” laboratories, always something of a fiction, has become a sick joke. Such funds as all but the best-blessed universities receive are used to keep body and soul together.

The research community is not entirely free from blame. The research councils, jealous of their autonomy and proud of representing some part of the research community, have shamelessly followed fashion as dictated by the British Government. If the word goes out that engineering is neglected, the Science Research Council obligingly adds “and Engineering” to its name. If Mr Kenneth Baker, the information technology minister, has a rush of blood to the head, funds are chiselled from budgets already under strain to provide new graduate courses and earmarked grants for research in information technology.

A similar complaint can be levelled at the universities. The threefold increase of student entry in the past two decades has not prompted the careful examination of how students should be taught that reason would require. Just as undergraduates are ill-prepared for working lives in the real world by the notoriously paradoxical and uniquely British blend of specialism and detachment, so the standard British preparation for a career in research (a PhD course), consisting of giving a young person a research problem and telling him to come back when it has been solved, seems ever more amateurish.

Some say that, by pointing to these and other failings of the research enterprise in the past few months, *Nature* has been disloyal to the British part of its constituency. But so long as there are steps the research community might take to defend itself against mediocrity, the opportunities should be recognized.

Impatience

The true culprit is in any case the British Government. Money is not the only problem. Although the science budget has been stagnant for nearly fifteen years, the reasons, when properly explained, are understood. Except for Mrs Shirley Williams' spell at the Department of Education and Science between 1976 and 1979, successive governments have made plain both their impatience with the research enterprise and their inability to understand that its needs are as much psychological as material. The now-constant harping on the need to conjure prosperity from research would give less offence if it less obviously implied that ingenuity is like water which can be diverted in one direction or another as in an irrigation channel.

The five years of the present administration have been disastrous. The government has been playing cat-and-mouse with the universities. At the end of the year in which Mrs Thatcher promised to protect the science budget, Mr Mark Carlisle decreed the reduction of university incomes by between 8.5 per cent and 13.5 per cent on the basis of a calculation of student demand for higher education now acknowledged to have been spurious (see *Nature* 19 July, p.175) and then promptly vanished from the scene. His successor, Sir Keith Joseph, who seems to be an earnest man, has been learning about education more empirically, and at the expense of other people's peace of mind. Mr Carlisle's promise of “level funding” has been followed by threats of continuing reductions, and academics whose place is at the laboratory bench have been assembled once more into committees to plan for a parlous future. Yet the big questions about higher education (the binary divide for example) remain untackled.

The government does not appreciate that continuing uncertainty saps the spirit. What seems (to the government) to be “keeping up the pressure” on a set of institutions regarded as unproductive and self-interested is a recipe for their permanent

debilitation. Four years ago, British universities might have benefited from a short period of upheaval. But the agony has been dragged out, by continuing parsimony and the government's indecision. The quality of British higher education is being debased by attrition.

The science budget is another kettle of fish, the Prime Minister's direct responsibility. So much was made clear in 1982. And it was Mrs Margaret Thatcher herself who refused the request for extra funds last year with the remark that “£500 million is a lot of money”. So it is. There are also circumstances in which it might be sufficient. But they do not now obtain in Britain.

The most obvious impediment to the survival of the British research enterprise is its legacy from the past. Research councils are lumbered with many research institutes or facilities built for the general use of the research community in happier times. The managers of these enterprises, if free agents, would no doubt prefer to start again, from somewhere else. But they are trapped between the knowledge that closing down a modestly worthy group or institution may be a national disservice and the wish somehow to embark on new adventures. There may be lessons for the future to be learned from all this.

The British Government's insensitivity to this dilemma is feckless. £500 million is a lot of money, but the government behaves as if its obligations are discharged once it has written the cheque. When currency fluctuations mean that the real cost of international subscriptions is unexpectedly increased, the research councils have to plead with the Treasury for partial help. When government departments cut back on their research commissions, taking income away from research institutes, nobody lifts a finger. If, for the sake of making room for new ventures, the research councils decide they must reduce their staffs, they know that they collectively must pay the costs.

Neglect

Indolently, the government does nothing even to consider the benefits that would accrue from redesigning the canvas on which its policy for research — civil, industrial and military — is at present drawn. Advisory committees continue to advise, but increasingly in a vacuum: specific advice (last year's recommendation from the Advisory Board for the Research Councils that the research enterprise needs more money) may be turned down flat, prescriptive advice (say on the proper relationship between industry and universities) may be published, briefly mentioned, discussed in journals such as this and then allowed to take its chances with the burden of public administration. Yet the British Government's total spending on research is ten times as great as the science budget proper. That a government apparently reconciled to expenditure on such a scale should be so indifferent to the uses made of it, but so vigilant in the control of marginal expenditure, is a recipe for providing an empirical demonstration that, in research, neglect can kill off even expensively supported enterprises.

But will not the transformation of the British research enterprise now being effected lead to stronger links with industry and to a more productive relationship between the two? That seems part of the government's justification. Starve the academics, and they will turn into industrial researchers. The flaw is that there is indeed a connection between discovery and application — and that the applications the British Government hopes for will probably flow from discoveries not yet made. So the outcome may be that which disappointed the dog in Aesop's fable, which dropped his bone to grab at its reflection in the water.

So what can be done to retrieve the situation? Sadly, the answer may be “Nothing”. Certainly, there can be no sure recipe for turning things around. As time passes, the chance of success further diminishes. More money is probably an ingredient of any rescue package. More important are an end to the harassment of the past five years, a willingness by the British Government to create a framework within which research may prosper and some sign that it understands what the whole enterprise is about. And speed. □

Non-proliferation treaty

United States asks for more safeguards

Washington

THE Reagan Administration, which in the past has taken a sceptical view of nuclear non-proliferation efforts, has had a change of heart. At a meeting earlier this month in Luxembourg of Western nations that sell nuclear equipment and materials, the United States pressed for adoption of a uniform requirement that all future sales be conditional on the purchasers' acceptance of "comprehensive" safeguards — meaning that all nuclear facilities in the country must be open to inspection by the International Atomic Energy Agency (IAEA).

The State Department indicated that no agreement was reached on the US request, but it seems to have been acknowledged that present safeguards are inadequate. The meeting was called, the State Department said, to allow the Western supplier countries to "compare notes" in advance of forthcoming meetings of IAEA on assurance of supply and the review of the Nuclear Non-Proliferation Treaty (NPT) scheduled to be held next summer in Geneva. Eastern bloc countries, which have participated in meetings of the so-called London Suppliers Group, were not invited.

The United States has very strict rules governing the export of nuclear materials. Unless waived by the President, the rules require that no country may purchase nuclear equipment, fuel or related materials unless it has comprehensive safeguards in place. Under NPT, non-weapons states must also accept comprehensive safeguards. But nothing in NPT prevents a signatory from selling equipment or materials to a non-NPT state that does not accept comprehensive safeguards. Suppliers are bound only to gain assurances that safeguards will apply to the particular items they are selling.

It is this loophole that the United States is now apparently trying to close. Several European countries, notably France (which has not signed NPT), have been extremely reluctant to restrict their markets for nuclear equipment. Purchasers who would be affected by the US proposal include Argentina, Brazil, India, Pakistan, South Africa and Israel, all of which have nuclear facilities (reactors, enrichment plants or reprocessing plants) that are not under IAEA safeguards.

Although President Reagan announced

his intention to press for the new uniform restrictions more than a year ago, there have been no signs of action until this month. Reagan did not raise the issue at the Williamsburg summit meeting, which came one month after the announcement, as many expected he would.

And within three months of the announcement, the Reagan Administration approved the sale of spare reactor parts to India, the transfer of heavy water to Argentina and the sale of computers and other equipment to South Africa. It has also arranged for third parties to supply nuclear fuel to India, South Africa and Brazil.

Stephen Budiansky

Pakistanis indicted

Washington

CONCERN over proliferation of nuclear weapons was underlined last week by the indictment of three Pakistani nationals in Texas on charges of attempting to export to Pakistan critical electronic components that, according to the indictment, could be used in the development of nuclear weapons. The Justice Department refused to discuss the case further.

Last month, Senator Alan Cranston (Democrat, California), released what he said was "substantial" new information about Pakistan's efforts to get the bomb. According to Cranston, Pakistan now has an operating uranium enrichment plant capable of producing 15 kg of highly enriched uranium a year, about enough to make one bomb. A reprocessing plant, based on designs that France supplied before finally cutting off assistance, is also in the works. (The head of Pakistan's nuclear programme, Dr A. Q. Khan, has been convicted by a Dutch court of stealing plans for the enrichment plant from the URENCO plant in Holland, where he worked.) Pakistan has an indigenous supply of uranium ore and a plant for converting it to uranium hexafluoride.

Although the reprocessing of plutonium from Pakistan's KANUPP power reactor is, in theory, a source of bomb materials as well, it would take essentially all of the spent fuel so far produced to yield a sufficient quantity for one bomb, according to Warren Donnelley of the Congressional Research Service. The KANUPP reactor is under IAEA safeguards, although some lapses — including chronic failures of monitoring cameras — have been reported. Cranston also said that China has apparently provided technical assistance to Pakistan on warhead design since the late 1970s. Stephen Budiansky

Sellafield

Cancer risk reviewed

IN November last year, a documentary produced by Yorkshire Television (YTV) claimed that the proximity of British Nuclear Fuel Limited's nuclear reprocessing plant at Sellafield (Windscale) to the village of Seascale in Cumbria could be a factor in producing cases of childhood leukaemia in Seascale. The Department of Health and Social Security set up an Independent Advisory Group chaired by Sir Douglas Black to assess the claims and its findings are published this week.

As the report admits, the claim that the reprocessing plant was a factor in causing leukaemia "is not one which can be categorically dismissed... nor is it easy to prove". There is, the report concludes, sound evidence for YTV's claim of an increased incidence of leukaemia in the village, which the report calls "unusual but not unique". But the number of children who developed leukaemia in a 30-year period at Seascale was less than 10 and there is some uncertainty about the true size of the relevant population.

Sir Douglas criticizes existing state bodies that monitor environmental pollution in Cumbria, chiefly the Ministry of Agriculture, Fisheries and Food (MAFF) and the National Radiological Protection Board (NRPB). There is, he says, evidence of a lack of coordination and he calls for a far more thorough investigation of cancer incidence in the region.

For West Cumbria as a whole, however, the mortality for childhood cancers is close to the national average. The report finds no conclusive evidence of any general risk to health for children or adults living near to Sellafield when compared with the rest of Cumbria. To those living in Sellafield who are worried about the cancer risk Sir Douglas offers "a qualified reassurance".

Perhaps the major recommendation of the report is the call for tighter government controls on the activities of British Nuclear Fuels Limited (BNFL). It is recommended that there should be formal consultation between BNFL and area health authorities over the possible health consequences of discharges, and particular stress is placed on the need to remove solvents from the discharges. The waste from Sellafield contains higher levels of emitters of alpha as well as beta and gamma radiation than that from similar installations in other countries, and the report recommends a critical review of the reasons for this.

BNFL itself, however, has made moves to "clean up" Sellafield, and weeks before the publication of the Black report announced plans to reduce discharges to a level "as close to zero as possible". □

**Investigation of the Possible Increased Incidence of Cancer in West Cumbria, Report of the Independent Advisory Group (HMSO £6.70).*

Correction

IN the article on the Dutch research council, ZWO, in *Nature* 309, 497-498 (1984), the space research institute's budget for the building of instruments was given as Dfl 50 million. It is in fact Dfl 15 million. □

Japanese research

New money for cancer

Tokyo

JAPAN'S "ten-year programme for cancer control" has begun to take off with the announcement by the Ministry of Health and Welfare (MHW) last week of details of six major research projects. New funds — around 4,500 million yen (£14 million) this year — are already being put to use and will become fully available in October.

The ambitious new research campaign is being conducted jointly by MHW, the Ministry of Education, Culture and Science (MESC), and the Science and Technology Agency (STA), with MHW as overall coordinator. Its inspiration comes partly from the soaring incidence of cancer — now responsible for around 24 per cent of all deaths — and partly from Prime Minister Yasuhiro Nakasone's enthusiasm for the life sciences, see also *Nature* **249**, 187; 1972).

The new funds come on top of expenditure for cancer research of around ¥1,800 million for MHW and ¥2,000 million for MESC. But the big difference is that about half of the new money is for "personnel" rather than research grants: there will be new fellowships, travel grants and special "research resident" fellowships in hospitals and, in the most radical departure from tradition, new opportunities for foreign scientists to work in Japan. This last move signals the confidence of Japanese scientists that their basic research is now as good as that in the West. MHW alone hopes to employ some 30 scientists from abroad for 1-3 year periods, mostly at the National Cancer Centre Research Institute.

To make the new system work flexibly and to get away from government bureaucracy, MHW is spending its ¥1,500 million share of the new money through a new Foundation for Cancer Research and additional funds are now being sought through appeals to industry. The foundation is also to sponsor visits from foreign lecturers; Paul Marks, president of New York's Sloan-Kettering Institute, will be one of the first.

Each of the three organizations will pursue its own cancer research programme but those of MESC and MHW are closely coordinated. The STA programme is more concerned with research "support technologies". The new MESC-MHW programme is divided into six main projects, each looked after by scientists from both ministries: on the MHW side, overall leadership comes from the National Cancer Centre Research Institute and its director Takashi Sugimura. Research will be carried out at universities, government research laboratories and hospitals throughout Japan.

In the first project, there will be intensive studies of oncogenes, where recent rapid advances had at first left Japanese

researchers rather behind. The situation has now been remedied, Professor Sugimura believes; his institute too is to have a new division especially devoted to the study of oncogenes.

The second project, on cancer-causing viruses — especially hepatitis B virus and adult T-cell leukaemia (ATL) virus — will tap some fast-moving research on the occurrence of ATL in the island of Kyushu.

Native research also features strongly in the third project which will search for new carcinogens and examine the mode of action of promoters and initiators of cancer.

Japan's proven skills in electronics will contribute to the fourth project's aim of developing better early diagnosis procedures. Digital radiography techniques have already reduced X-ray exposures in mammography to one-tenth of former values and have the potential to make mass

screening totally safe. New state of the art electronic technologies are the next objective — extra-small endoscope television cameras, cell diagnosis equipment capable of distinguishing cancer and normal cells and a capsule which can be swallowed and will then radio such parameters as stomach pH and enzyme activity.

The fifth project will look at new cancer treatment methods including the use of prostaglandins and activated vitamin-83, both of which have shown some promise as anti-cancer agents. The possibility that drugs that knock out the activity of the products of oncogenes may be effective in cancer treatment will also be pursued. Radiation therapies with new types of particles will continue to be supported.

The last project aims at understanding the immunology of cancer and investigating the use of interferons, interleukins and other immune substances in curing cancer. Monoclonal antibodies will also be studied both for their value in early diagnosis and in the hope of constructing "magic bullets".

Alun Anderson

UK oceanography institute to close

THE Taunton site of the Institute of Oceanographic Sciences (IOS) will be closed down in mid-1985 as a result of a decision by the Natural Environment Research Council (NERC). The intention is that the two main areas of research traditionally carried out at Taunton — waves and sedimentology — will be strengthened by moves to the other IOS sites at (respectively) Wormley and Bidston, where related research is already carried out. NERC officials acknowledge that short-term damage to current research may result.

Deliberations on the future of IOS started last year (*Nature* **306**, 102; 1983), partly in the context of economic pressures on NERC but also with a possible view to rationalizing the presently dispersed institute (Bidston is in north-west England while Wormley and Taunton are in the south and south-west respectively). It was eventually decided that the development of a single institute would be too costly, and in terms of the construction of new accommodation, an expanded Bidston site offers the cheapest option.

However, a spokesman for NERC emphasized that the financial savings that would follow the move are not significant — at least in the short term — and that scientific considerations provide the ultimate rationale. Research at Taunton on waves will be amalgamated with other work at Wormley on the oceanic upper boundary layer. The sedimentology now under way at Taunton will move to Bidston, where research on continental shelf water dynamics is already carried out. Indeed, the new move is seen by some as the effective establishment of a distinct continental shelf division of IOS, strength-

ening the Bidston site.

The uncertainty following last year's announcement of a review of the IOS sites has already increased the rate of staff departures from Taunton, whose personnel have decreased in number by 25 per cent over the past year. Other staff are known to be looking for jobs or are tied to the area for family reasons. However, NERC's secretary, Dr John Bowman, said that, in his experience, more people move to new sites in such circumstances than initially expect to do so, and that putting together groups of researchers in related fields would help to stimulate research.

The IOS closure occurs in a changing context of university research in the United Kingdom. As a result of a University Grants Committee initiative, two university oceanography departments (Southampton and the University College of North Wales at Bangor) are to be significantly expanded. The intention is that the strengthened Bidston site will be able to collaborate with Bangor and with marine science and technology expertise at Liverpool and Manchester for example. The Bidston laboratory will also carry out commissioned research for government departments on the sedimentary fate of plutonium discharged from the Sellafield nuclear waste processing plant.

The director of the Taunton site, Mr M.J. Tucker, was less than overjoyed at the news of the closure. "While I see that there may be long-term benefits in an expanded site at Bidston, the short-term cost will be significant. The aim must now be to grasp the nettle and stimulate people's imaginations with the idea of working at a centre devoted to continental shelf problems."

Philip Campbell

US space station

No enthusiasm in Congress

US PLANS for a permanent manned space station, now being pursued enthusiastically by the National Aeronautical and Space Administration (NASA), have failed to impress Congress's Office of Technology Assessment (OTA). In a report on the ambitious plan to be published next month, OTA says it can find no "compelling, objective, external case" either for the space station options now being considered or for the likely cost to the public.

That many industrial companies have expressed an interest in participating in the project has however convinced OTA that there is a "persuasive" rationale for some sort of permanent space infrastructure, which is seen as essential if the United States is to retain its leadership in civilian space activities. The Soviet Union has, it notes, made a commitment to the permanent occupancy of space and has more experience with orbital stations. More than a hundred conceptual uses have been identified which have potential for science, technology and commerce, and individual companies and countries have made clear their desire to play a continuing role in the utilization of the space station's facilities. But, says OTA, there is no need in the near future for all the station elements advocated by NASA.

Among the uses for a space station accepted by OTA are "sophisticated" experiments in life sciences and materials sciences and trials of new instruments and activities. The station would also permit fuel and supplies to be warehoused in low Earth orbit, so allowing more efficient staging of voyages to higher orbits and Solar System destinations as well as repair and maintenance of complex satellites. But OTA is concerned that sufficient resources should be made available for development of these activities, otherwise "the rationale for the infrastructure vanishes". Some of the activities, OTA notes, would certainly need an on-board human workforce, if not a permanent crew: automated facilities for all the activities envisaged are unlikely to be available before the year 2000. But OTA concludes that it is impossible to define exactly what is required until a national consensus has been reached on objectives.

A range of possible objectives considered by OTA produces cost estimates for different options ranging from well below to well above the projected NASA proposals. Although finding sources of finance is unlikely to be a fundamental constraint on the programme, OTA seems to be suggesting that by adopting an "economy model" approach to the space station, the drain on federal funds could be very much reduced. Private companies may well be used to develop the free-flying

platforms envisaged by NASA, for example. NASA's plans are likely to cost \$8,000 million for "initial operating capability" or up to \$20,000 million for the full version.

Besides accusing NASA of not having properly thought out its technical objectives, OTA suggests that NASA's plans have been influenced by the belief that the agency could not long survive in its present form without some new large space programme: a series of less grandiose schemes would, OTA suggests, not have provided NASA with the same guarantee of long-term funding. But OTA wants NASA to stand aside wherever possible for private industry, and concentrate instead on the "very best of space-related science". Rather than a development effort aimed at producing the best designs, OTA wants a pragmatic ethic to dominate the process: programmes should be thoroughly defined in advance and deadlines should not be allowed to slip;

existing technology should be used except when this is clearly impossible.

OTA also has some interesting reflections on policy for international cooperation. It notes that the United States really has no need of such cooperation and could perfectly well develop a space station on its own if this suited. Furthermore, any sort of international cooperation is bound to be a possible source of friction and conflicting interests: the report notes that negotiations with the European Space Agency have already highlighted the potential for differences of opinion over how the project should be managed. Despite the problems, however, a cooperative approach would seem to be advantageous: the United States would prefer to be the dominant partner in space exploitation over the next few decades, and "locking in" its most likely competitors for dominance into a US-led system would go a long way towards achieving this goal.

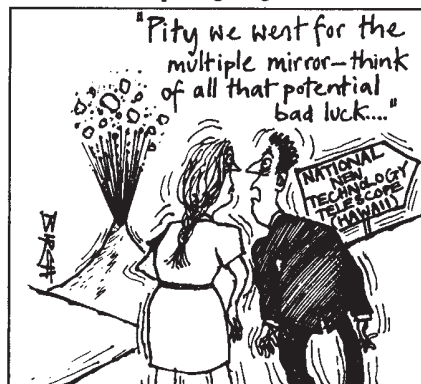
Tim Beardsley

Telescope for 1990s

Multiple choice on mirrors

Washington

THE United States' premier ground-based optical telescope in the 1990s and beyond, the National New Technology Telescope, is now almost certain to be a multiple mirror instrument. A scientific advisory committee to the National Optical Astronomy Observatories (NOAO) has decided in favour of the multiple mirror option rather than the competing segmented mirror



design and NOAO is expected to approve the choice in August after a full technical report has been completed.

The decision was by all accounts a difficult one. Although either would have been feasible, the multiple mirror design seems in the end to have won primarily because its instrumentation was less likely to be a limiting factor in use. The telescope will have four 7.5-metre mirrors in a square configuration, giving a light-collection rating equivalent to a single 15-metre mirror but a baseline aperture, limiting in infrared observations, of 21 metres. Although the multiple mirror design calls for more detectors, construction of instruments such as spectrographs will be simpler.

Apart from producing the mirrors, a major challenge will be in aligning them with sufficient accuracy for use in combined beam mode, for which the light paths have to be identical in length. One factor in the minds of some committee members seems to have been that even if the alignment does prove to be a problem, there is still a lot of science that can be done with the four mirrors separately. If there are comparable problems with a segmented mirror design, however, the result could be an expensive white elephant. Either design is likely to cost more than \$100 million. But Dr Jacques Beckers, who heads NOAO's advanced development programme believes the alignment problem can be overcome.

The multiple mirror design will have a wider field of view than the segmented mirror could reach, and will therefore be better for such uses as multi-object spectroscopy. Wide-field direct imaging, on the other hand, will be less well served. The complex diffraction pattern of a multiple mirror will make disentangling separate faint sources more difficult, and many observations will take longer. The clinching factor, by one account, was that the multiple mirror design produces images that are more compatible with pixels of charge-coupled devices; having too many pixels per star introduces a new kind of error.

After NOAO has formally approved the design concept, work will begin on detailed technical proposals. A submission for funds to the National Science Foundation would follow in a year or two. In the meantime, studies will continue into a possible site for the telescope, likely itself to be a contentious affair. The two possibilities now being considered are Mount Graham, in Arizona, and Mauna Kea in Hawaii.

Tim Beardsley

Assisted fertilization

Legal restraints proposed for UK

THE much-advertised report of Dame Mary Warnock's committee on human embryology, published last week*, has surprised those working in the field by advocating criminal sanctions to enforce the regulations it recommends (see also p.269). The committee would outlaw most forms of surrogate motherhood as well as studies of human embryos after the fourteenth day beyond fertilization. All research with human embryos *in vitro* will have to be approved by a committee to be appointed under statutes not yet enacted.

The committee has been able to reach agreement on most issues dealt with by acknowledging that many of its recommendations may seem unduly lax to many sections of the community, but that they are justified by their relevance to beneficial medical treatment, chiefly of infertility. The committee has thus side-stepped contentious questions such as abortion and has replaced the question of when life (or "personhood") begins by the ethical and legal question "how is it right to treat the human embryo?" The committee points out that practices that might be justified by a utilitarian calculation of costs and benefits may nevertheless be morally unacceptable and that a society with "no inhibiting limits" would lack moral scruples, which "nobody wants". The committee considers its recommendations of legislation the "minimum requirement for a tolerable society".

The treatment of infertility by new techniques is wholeheartedly supported by the committee's report, which rejects objections of principle and on grounds such as the need to restrict population growth. It advocates the more systematic collection of data on the incidence of infertility and (in Britain) the provision of infertility services in the next round of capital development plans for the National Health Service. No firm opinion is offered on the provision of help with childbirth for female homosexuals.

To the extent that the new developments involve donors (of sperms or ova) and recipients, the committee asks that the identities of both parties should not be disclosed to each other. But where a child is not the exclusive genetic offspring of its rearing parents, the committee would allow (on majority at eighteen) limited information about the genetic parent (ethnic origin, genetic well-being, etc.).

An important part of the committee's report is intended to bring in from the shadows the practice of artificial insemination by donor (AID), by which at least 1,000 pregnancies are known to have been achieved in Britain in 1982. The committee advocates an arbitrary limit of ten children fathered by a single donor.

The practice of *in vitro* fertilization is commended, but within a framework of

licences and in the knowledge that the overall success rate is still low.

By symmetry, the committee would apply the same principles to the transfer of a fertile egg from one woman to another, but says that the use of this technique in the treatment of female infertility is likely to be limited because eggs cannot now be frozen successfully. Donated eggs are therefore more likely to be used for the production of embryos *in vitro*.

Warnock's law

ENFORCEMENT of the Warnock committee's proposals would rest with a statutory licensing authority on which there would be "substantial lay representation", with a lay chairman in charge. The committee would license practitioners providing treatment, license researchers and also approve individual investigations. The commission would also issue guidelines on the kinds of experiments likely not to be approved and would superintend the working of the legislation and the collection of data. Breaches of the terms of a licence would be criminal offences.

The proposal that practitioners should be licensed will effectively make AID a regulated service. AID births would also formally be legitimized (and so would not formally require adoption by the mother's husband), while donors' rights and responsibilities would be legally extinguished.

Organizations seeking participants in surrogacy would be banned, and those operating them, together with "professionals and others who knowingly assist in the establishment of a surrogate pregnancy", made liable to criminal prosecution.

Warnock says that the licensed use of frozen embryos should be permitted. The status of frozen semen (and ova) should be reviewed after five years when, if the donor cannot be traced, responsibility should pass to the storing authority. Frozen embryos should not be stored for more than ten years, and the committee says that there should be no rights of ownership in embryos but only a "right to use or disposal". This should pass to the survivor if one of the genetic parents should die and to the storage authority if both die. In default of agreement between genetic parents the storage authority would again step in. Embryos not *in utero* at the death of a genetic father should be "disregarded for the purposes of inheritance and succession".

On the regulation of research, licensing is again the guiding principle. The committee notes that consistency requires the same regulations for the use of fetal material, in Britain at present regulated only by an official code of practice.

Warnock draws a firm line at the practice of surrogate motherhood where the mothers are not intended to keep the child, chiefly because of emotional and legal difficulties but also because of fears of "commercial exploitation".

Apart from the treatment of infertility, the committee says there is a role for the use of donor eggs and sperm in the avoidance of hereditary disorders. It is, however, "dubious" of the widespread practice of gender determination except where the objective is to avoid sex-linked genetic disorders. The report even recommends that "do-it-yourself" sex-determination kits should come within the framework of the UK Medicines Act, which regulates the safety, efficacy and sale of drugs and other medicaments.

On research with *in vitro* embryos, the Warnock committee has sought a balance between the view that embryos are human beings from the point of fertilization and that which holds that the potential benefits of research are substantial, especially in relation to genetic disorders which are uniquely human. It has therefore accepted the view that even the earliest human embryos should be accorded "a measure of respect" greater than that due under the law to animal embryos, and recommends that research should be allowed only under licence, and subject to detailed approval of experimental protocols. The proposed time-limit of fourteen days from fertilization is chosen as that at which the neural streak is thought to appear in the developing human embryo. Research, the report says, should be carried out wherever possible with the consent of the genetic parents.

Of the more futuristic possibilities, the committee would allow experiments in cross-species fertilization, but embryos would have to be destroyed after the first cell division. The uses of embryos for testing drugs may be permitted in circumstances to be decided by the licensing authority, but the transfer of human embryos to the uteri of other species would be banned outright. The report says that cloning is too far ahead to require legislation. The committee thinks it unlikely that the practice of using a few cells from an early embryo will be used for genetic diagnosis so long as the success rate expected for the birth of a full-term child from the remainder of the embryo (which would have to be frozen pending the outcome of the diagnosis) remains small.

For such a contentious subject, outright expression of dissent from the majority view are few. The members of the Warnock committee insist there may be a place for surrogacy in special circumstances. Three reject experiments on human embryos, while four say that research should not be permitted on embryos created for that purpose. □

*Report of the Committee of Inquiry into Human Fertilisation and Embryology (Cmnd 9314, HMSO, £6.40).

UK research

Catalogue of scarcity

THE ability of the British academic community to undertake fundamental research to international standards is being seriously damaged at all levels. Moreover, older researchers are stopping work prematurely while younger members are leaving to work abroad in abnormal numbers.

These are two conclusions of a detailed study of the research grants awarded over the past few years by the Science and Engineering Research Council (SERC) which highlights the significant proportion of grant applications, judged by peer review to be of the highest calibre, that have had to be turned down for lack of funds.

As well as collating and analysing statistics of SERC's grant awards, the steering group that prepared the report commissioned six consultants to visit research centres and monitor the peer review process. The report concentrates on the activities of the Science Board (which supports research in physics, chemistry, biology and mathematics and related fields) and the Engineering Board, touching only briefly on the Astronomy, Space and Radio and the Nuclear Physics Boards.

Grant proposals to all the boards are graded by SERC into three categories: alpha (to be supported "at all costs" for their scientific merit and, in engineering, for their potential industrial applicability), beta (sound science to be supported if money is available) and gamma (to be rejected). Until the financial year 1978-79, all alphas and some betas were supported. Since then, some alphas have been turned down every year, even though the alpha standard as applied in SERC's peer review process has risen during the period of the study. Predictably, the number of grant applications as a whole to SERC has risen as other sources of funds have diminished, particularly for chemistry, physics and biology.

The report says that further strains have arisen from the significant decline in the support for university departments by the University Grants Committee (UGC) under the "dual support system". As a result of the substantial decline in UGC's general grant, academic staff have heavier teaching loads, thus increasing the need for SERC to support research staff.

The statistics show that, although the demand for grants has varied considerably between disciplines (and the number of grants recommended for funding in the

alpha category roughly *pro rata*), more than 20 per cent of alpha applications in most subject areas in 1982-83 were left without support.

Care is required in the interpretation of the figures because some of the applications (about 10 per cent in some fields) are alpha-rated proposals resubmitted from previous years, but the report emphasizes the dangers of the resulting delays in competitive fields. It also says that the trends after adjustment to 1982-83 prices underestimate the purchasing power of earlier

periods because salaries have risen faster than inflation.

In many areas, the ability to fund alpha proposals is reported to have diminished further in the current year. The report concludes that the council's capacity to respond to proposals for high quality research — particularly unsolicited applications containing novel ideas that might lead to significant developments in the future — is being seriously undermined. The report recommends the immediate allocation of extra money, particularly for unsolicited proposals; it also recommends that the movement abroad of researchers be monitored systematically.

Philip Campbell

Where the cuts fall

Details of the impact of budget shortages in different areas as follows:

● **Physics.** Following the Vinen report in 1982, the Science Board attempted to offset a perceived decline in the activity of the UK physics community. Nevertheless, although the demand for grants has risen steadily, the available funds have decreased by 9 per cent in real terms so that, by 1982-83, the proportion of alpha applications refused had risen to 28 per cent by number. Types of proposals particularly affected have included those representing changes in direction of research groups or new ideas coming from exceptionally able younger individuals.

● **Chemistry.** In contrast with previous years, the chemistry community is almost entirely dependent on SERC for financial support, so that the number of applications has increased significantly. In 1982-83, the proportion of alpha proposals unsupported was 29 per cent. According to the report, about 40 per cent of the chemists whose alpha-rated applications were refused afterwards abandoned them completely. Problems have arisen particularly when sophisticated instruments such as mass spectrometers, which can be of use to a department as a whole, become essential but cannot be provided. The report points to a trend of opting out, early retirement or emigration to Europe.

● **Biology.** Although the biological sciences are supported by all four science research councils, as well as outside sponsors, SERC is expected to support fundamental research which other sources (devoted to a more applied emphasis) will not provide for. In the context of a steadily increasing demand, the proportion of alpha proposals unfunded in 1982-83 was 17 per cent. The report highlights a significant number of researchers leaving the United Kingdom. It also points to the "sophistication factor" as a result of which, for example, the demand for costly nuclear magnetic resonance equipment has risen considerably. Small departments suffer particularly when SERC can provide only part of the cost of a major piece of apparatus, and the balance cannot be found.

● **Mathematics.** In 1982-83, SERC supported about 5 per cent of the mathematics community, UGC providing the majority of the balance (as is traditional). But the demand has been rising, partly because of the increasing use of computers by pure mathematicians. The proportion of alpha proposals unfunded had risen sharply by 1982-83 to 49 per cent.

● **Materials Science.** A rise in demand of 42 per cent since 1980-81 has been accompanied by a continuous fall in support, with the result that the proportion of alpha applications unfunded in 1982-83 was 43 per cent. The report also says that, while many researchers are able to tailor unsuccessful proposals for industrial applicability and obtain alternative support, this trend is adversely affecting fundamental research.

● **Engineering.** As with materials science, the unrequited alpha problem has, according to the report, been compensated for to some extent by industrial support at the cost of more fundamental research. The average engineering grant request (£36,000) exceeds that in science (£27,000) mainly because of higher demand by the engineers for technicians and research assistants. The consultants say that this is justified. Over the past decade, SERC has given particular encouragement to engineering and it is only very recently that the alpha problem has emerged. The report predicts that the number of combined (CASE) grants jointly sponsored by industry and SERC will decrease.

● **Astronomy, Space and Radio Board.** In 1982-83, the proportion of alpha proposals not supported was 17 per cent, but in the previous two years it had been about 24 per cent. The report points out that the ASR board has a number of large-scale continuing grant commitments which squeeze other applications, significantly increasing the proportion of ordinary alpha grants unsupported.

● **Nuclear Physics Board.** Although no details are provided, the steering group reports that the problem faced is comparable with that of the ASR board.

Philip Campbell

Steering group members

Professor Sir Jack Lewis (Chairman) (University of Cambridge).

Professor P J Lawrenson (University of Leeds).

Professor W L Wilcock (University College of North Wales, Bangor).

Dr W L Wilkinson (British Nuclear Fuels Ltd).

Policy on heritability of IQ

SIR — *Nature* has recently given much attention to the controversy about the heritability of IQ. Beardmore and Karimi-Booshehri¹ was followed by Teasdale and Owen² and the leading article (14 June, p.519) in which you make the point that fears that resolution of the nature-nurture debate may drastically affect policy are likely to prove unfounded, and that there is everything to be gained in terms of our understanding of how the human mind works.

While few will deny that whatever it is that goes by the name of intelligence must have some basis in physical reality, I find it difficult to share your optimism that the determination that some part at least of human intelligence is heritable will bring us dramatically closer to an understanding of the functioning of the mind. The neurogenetics of man is (I hope) still a long way off, while mere recognition of the fact that genes seem to be somehow involved does not appear to bring us much nearer to such an understanding of the organization and "mechanisms" underlying higher mental functions.

What, on the other hand, is there to fear from the results of research into the heritability of IQ and other presumed measures of ability? Your argument that whatever research turns up should be faced with equanimity simply as more new and interesting information springs, no doubt, from a conviction that science must flourish without restraint and no area of research must be suppressed or wilfully ignored. In the long run, knowledge of the truth can only be to the good. Unfortunately, however hard I try, I find it difficult to discover the good in IQ heritability and allied lines of research. For it is neither truth nor reason that determines how the results of such research are applied. IQ research has been and will continue to be abused. Such abuse so far has shown the scantest respect for trivial matters such as scientific veracity. Profound conclusions have been drawn from inconclusive data and these conclusions, far from being mere bones of academic contention, have been seized upon and paraded in support of the most obnoxious doctrines and the most inhuman ideologies. Never mind that the issue has never been settled; if ever it is, so much the better; if not, we will just make do with distortions.

Is it not rather naive to suggest that, just because the outcome of IQ research ought not reasonably to influence social policy, it will not in fact do so? And let there be no doubt that it has. Nobody need be reminded of Nazi genocide — suitably buttressed by obliging scientists (worthy successors to the eugenics enthusiasts of slightly earlier times). Forty years later, fascist ideology is still alive and well — witness the correspondence in *Nature* as a

sequel to the appropriation of the selfish gene concept to their ideology by the neo-Nazi National Front of your country about two years back.

And if anyone should look upon these things as aberrations that have had their day (which they are not), then there are other examples of how social policy and attitudes have been informed by misinformed science. Thus US immigration and military recruitment officials have in their time found inspiration in ethnically biased studies and writings on IQ. Racism draws comfort from learned though unestablished work claiming to discover the superiority of brains encapsulated in fairer casings (too irksome, though, the Japanese anomaly), and while Western governments shudder at the mention of apartheid, South Africa carries on with impunity — and tacit support, as its trade record will show. Not to be left behind as science surges ahead, Singapore has recently launched a programme scientifically to improve the quality of its population: graduates are to be given economic incentives to join hands with fellow graduates and raise large families, while the inferior non-graduate genes are to be kept at bay through incentives for their bearers to get themselves sterilized. Perhaps the Singapore Government has access to scientific knowledge that has not yet permeated elsewhere; at any rate the government seems impervious to the logic of why scientific findings on the heritability of measures of ability (in this case, graduation) are not likely to influence social policy.

What, then, about research on the heritability of ability? Should it be proscribed? No, but tolerance is one thing, espousal quite another. If claims are made about the heritability of human attributes of mind or personality, let us be very critical; let us even be suspicious, let us trail one step behind rather than rush recklessly ahead. Perhaps in the future, when man has come to organize his society rationally, justly and humanely, we can face questions about ourselves with less trepidation about how they might lead us into self-abuse. At this stage in the history of our species, I find myself hard-pressed to share anyone's enthusiasm for this particular aspect of human self-enquiry. The price of half-knowledge has already been terrible; if we would know more, we must have the maturity to handle our knowledge.

J. MANJREKAR

*Tata Institute of Fundamental Research,
Homi Bhabha Road,
Bombay 400 005,
India*

1. Beardmore, J.A. & Karimi-Booshehri, F. *Nature* 303, 522 (1983).
2. Teasdale, T.W. & Owen, D.R. *Nature* 309, 620 (1984).

More visa trouble

SIR — I would like to draw attention to the following problem. On 27 August 1984 in Prague, Czechoslovakia, the 6th General Conference of the European Physical Society (EPS) will begin, at which about 1,400 participants are expected. I am one of them. Although I have registered, paid all the conference fees, obtained an official invitation from the local organizing committee and applied for a visa, the Czechoslovak Embassy in Stockholm informed me on 6 June 1984 that "the respective Czechoslovak authorities did not approve" the issue of a visa.

This is a severe violation of the freedom of scientific exchange. The situation is, however, aggravated by the fact that during the Prague conference, several decision-making bodies of EPS are scheduled to meet. Since I am an elected member of the EPS Council (one of the 9 representatives of 2,500 or so individual ordinary members of EPS), the decision of the Czechoslovak authorities prevents me from fulfilling my duties.

The European Physical Society was founded in 1968 by, among others, Professor Gilberto Bernardini from Pisa, who endowed it with the spirit of the great European humanitarian traditions and principles of tolerance, mutual respect, respect for individual rights and freedoms, equality, openness and democracy. Can the society and its members now remain silent?

In discussing and trying to solve my visa problem with various EPS bodies and colleagues, my attention was drawn to the booklet *Advice to Organizers of International Scientific Meetings*, prepared and published by the Standing Committee on the Free Circulation of Scientists of the International Council of Scientific Unions (of which the Czechoslovak Academy of Sciences is a member).

The ICSU booklet lists measures that may be relevant in such situations: publicizing information concerning the refusal to grant visas; issuance of a formal protest statement at the beginning of the meeting; withdrawal of international sponsorship; possible cancellation of the meeting or removal to another site; issuance of a statement that decisions of the meeting cannot be considered valid until all voting delegations, including those not allowed to be present, have the opportunity to exercise their voting right; independent decision on the part of some scientists voluntarily to absent themselves; recommendation to all ICSU bodies not to accept invitations to hold meetings in the country until the situation is remedied.

It is of course a matter of conscience for those planning to go to Prague which, if any, individual decision they will adopt.

FRANTISEK JANOUCH

*Research Institute of Physics,
S-104 05 Stockholm,
Sweden*

Artificial fertilization made natural

Britain has been given a framework for new laws on in vitro fertilization. But the Warnock package is unduly inflexible, while its appearance will stimulate demand, thus changing the circumstances.

THE most surprising consequence of last week's report of the Warnock committee is that, in Britain at least, *in vitro* fertilization will be a little more respectable (see p.266). The committee says that one of its objectives, given the disagreements among its members and their changing views, is to contribute to a high-level public discussion. By that test, the exercise has been a success. The report is also succinct and literate. The temperateness of the Warnock view will help to cool the general opinion of *in vitro* fertilization and related techniques. By putting the most perplexing future uses of *in vitro* fertilization and embryo storage in an orderly framework, the committee has helped to make the techniques themselves seem reasonable.

This, unfortunately but unremarkably, does not mean that everything in Warnock is as it should be. But critics should themselves accept the committee's starting point — the argument that in a pluralistic society, it is unreasonable that the wishes of a minority should prevent others from following what seems to them a reasonable course but that, equally, to order society as if dissenting views are of no account simply because they are those of a minority is to ask for trouble. The Warnock package, admittedly a compromise, satisfies this criterion.

The flaws in the package are also clear. Regulation of some kind has for years been necessary to deal with AID (artificial insemination by donor). Physicians, faced with the prospect of government legislation, will be kicking themselves that they have not already regulated themselves. The ferocity with which the majority of the Warnock committee would prohibit surrogate motherhood is uncharacteristic: why should a childless woman with a hysterectomy be denied the chance of a child with the willing help of another woman, perhaps a sister? The difficulties the Warnock committee identifies, confusion about parenthood on the death of the genetic father, for example, are real enough, while the emotional stress of the surrogate mother after a successful birth is probably unavoidable. But the proposed ban would make bad law, inconsistent and unworkable. Some cases of surrogate motherhood (to be banned) would be distinguishable from donations of frozen embryos (to be allowed) only by the intentions of the participants, which might not be disclosed. Would the physicians involved then be prosecuted after the

event? Much better that surrogacy should be regulated professionally, with the requirement that each woman should be advised by a separate physician and that each surrogate pregnancy should be registered before completion.

The proposed regulations of AID are, by contrast, less stringent than they might be. Warnock assumes that sperm donors will be free from sexually transmitted diseases and will volunteer information about genetic defects. But these questions can now be settled objectively. It would have been helpful if the committee had said where it stands on the precision with which the genetic constitution of donors should be defined. Its opinion that users of sperm (and ovum) banks should not for the time being be given detailed information about donors is, however, unrealistic, as some of the committee recognize. The pressure from recipients will be too strong for physicians to resist. It would be better that the recording and analysis of genetic data on donors should be detailed and systematic, and that access (within the requirement of anonymity) should be open to legitimate enquirers — parents, offspring and researchers.

Most conflict on the Warnock proposals will however centre on the intention that research with embryos should be allowed. Those who hold that fertilized ova are human beings will be up in arms, and the strength of their position should not be underestimated. If the manipulation of intact embryos produced by abortion late in pregnancy is disallowed, on the grounds that they are too much like people even if they are not independently viable, it is at least formally inconsistent that younger embryos should not be so protected. The function of the arbitrary fourteen-day limit is to create a barrier between those extremes, for which purpose more or less any limit would suffice. The sensible licensing and scrutiny proposals should, however, serve the same purpose in meeting the general opinion that research with embryos should be seemly and deliberate. So why have both the licensing system and the limit? There is already strong reason to believe that investigations over a period of time perhaps twice as long would be of value in the avoidance of teratogenic defects. It is essential that potentially important investigations should not be made artificial by too inflexible a limit.

The proposed fourteen-day limit is

especially awkward in the British context. Warnock proposes that the limit should feature in the primary legislation, in which case it could be changed only by amending legislation. In the British parliamentary system, this is bound to be a serious obstacle to change. Governments' legislative timetables are always overcrowded, while the incentive to delay will be further increased by the fear that amending legislation will provoke a repetition of the original hot debate.

The British Government has another difficulty — the near-certainty that Members of Parliament will seek to anticipate the proposed legislation by piecemeal enactment of separate Warnock proposals. Private members' bills on surrogate motherhood are already on the stocks. The obvious danger is that piecemeal legislation will yield a piecemeal legislative framework leaving everybody in a muddle. Only speedy action by the government can avert this danger.

For the rest, everybody should recognize that the Warnock legislation has some of the features of a quantum measurement — the legislation will affect the situation it is designed to regulate. Merely defining a role for *in vitro* fertilization will increase demand for the service. Specifying the circumstances in which research with human embryos will be permitted will further stimulate legitimate interest among scientists. One result, among potential parents, will be the expectation that their children can with care be born with small risk of genetic defect. The Warnock committee probably underestimates the speed with which it will be possible to use DNA probes for the diagnosis of the genetic health of an embryo from a part of it, growing an intact child from the rest.

Inevitably, such concern will direct attention to the issue of eugenics, which has not recently been given the attention it deserves. If at some point it becomes common that in the course of avoiding genetic defect, some parents also seek to improve on what their own genes would produce, there may be great resentment among potential parents to whom these opportunities do not occur, or are denied. Yet in principle, there can be no persisting objection to constructive eugenic practices provided that they do not weaken the ties that normally bind parents to their children (one generation to the next). No doubt this is the agenda for the next committee on this kind of subject. □

Astronomy

Masers in the nuclei of galaxies

from James Moran

THE occurrence of cosmic masers, in which intense beams of partially coherent microwave radiation are emitted from gas clouds, is a curious phenomenon that is seen in our Galaxy in the dusty envelopes of both newly-formed massive stars and evolved stars with large stellar winds^{1,2}. Maser action in the emission from water vapour was discovered in 1969 by a group at Berkeley led by C. H. Townes, who also built the first laboratory maser³. Since then, more than 200 cosmic masers have been found in our Galaxy and a further 10 have been found in other galaxies. A dramatic new step in our understanding of cosmic masers has now been taken by Claussen, Heiligman and Lo who report, in this issue of *Nature*⁴, on a recent search for extragalactic masers. One of the galaxies they surveyed contains the most luminous water-vapour maser yet found and, intriguingly, their results implicate a novel source of excitation of powerful masers.

Observations with very-long-baseline interferometers (VLBI) that span two or more continents and have angular resolutions of about 300 microarc seconds⁵ have shown that a maser surrounding a newly-formed star consists of hundreds of bright spots having different velocities, equivalent blackbody temperatures of 10^{14} K or more and sizes of 10^{13} – 10^{15} cm. The cluster of spots is confined to a region of diameter less than 10^{17} cm. The strongest known maser in our Galaxy has a luminosity of about $1 L_{\odot}$ (4×10^{33} erg s⁻¹) or about 3×10^{49} photons per second.

Several years ago Churchwell *et al.*⁶ used the 100-m telescope at the Max Planck Institute in Bonn, FRG, to survey the nearby spiral galaxy M33 in search of water-vapour masers. This and subsequent studies detected eight masers similar to the ones in our Galaxy. Subsequently, Marques dos Santos and Lepine⁷ and Gardner and Whiteoak⁸ found powerful masers in NGC4945 and the Circinus galaxy, respectively, with much greater strengths ($160 L_{\odot}$ and $40 L_{\odot}$) than the strongest one in our Galaxy. These results did not seem particularly unusual because the observations were made with low-angular-resolution radio telescopes and, conceivably, masers from many newly-formed stars were contributing to the observed emission.

In their study reported on page 298, Claussen, Heiligman and Lo used a new low-noise amplifier and a wide-band acousto-optical spectrometer on the 40-m telescope of the Owens Valley Radio Observatory. They surveyed the nuclei of 29 nearby spiral galaxies and found four new water-vapour masers. Two of them are extremely luminous: NGC1068 has the most powerful maser yet found — with a

luminosity of $350 L_{\odot}$ — and NGC4258 has one with a luminosity of $120 L_{\odot}$.

The most surprising result of the study comes from observations of the maser in NGC4258 with the Very-Large-Array telescope. Claussen *et al.* found that all the emission was confined to a region having a diameter of less than 0.07 arc s, corresponding to a size of 2 pc. Now the 'pump', or source of excitation, of a cosmic maser is thought to be one or more massive stars. But the region measured in NGC4258 is too small to contain many massive stars unless they are packed with unusually high density. Claussen *et al.* suggest, therefore, that some other type of object may be exciting these powerful masers. This situation is quite different from that found in the galaxy IC4553, where a weakly inverted, but very extended, cloud of OH gas seems to be amplifying the continuum radiation from the galactic nucleus⁹.

As Claussen *et al.* point out, it is difficult to understand how these powerful extragalactic masers are pumped. Details of maser pumping cycles are complex, but general constraints can be placed on the power of the maser and the power of the pump source. Cosmic masers are assumed to be roughly spherical clouds that radiate isotropically. The radiated power is $h\nu n_1 \Delta p V$, where h is Planck's constant, ν is the frequency, n_1 is the upper-state population, Δp the differential pump rate and V the volume. The masing cloud is probably made up predominantly of H_2 gas, with a trace of H_2O ($n_{H_2O} \sim 10^{-4} n_{H_2}$), of which about 1 per cent will be in the upper state of the maser transition. The hydrogen density must be less than $\sim 10^{11}$ cm⁻³, or collisions will thermalize the H_2O population levels and quench the maser. A reasonable upper limit for n_1 is therefore 10^5 cm⁻³. The total pump rate, p , is limited to ~ 1 s⁻¹, a typical value of the Einstein A coefficients for transitions linking the energy levels involved in the maser. The volume of the maser, from VLBI measurements, is less than 10^{45} cm³. Hence, pump models with efficiencies, $\Delta p/p$, of ~ 0.1 can barely account for the strongest galactic maser. Furthermore, a cosmic maser usually needs at least one pump photon for every microwave photon. The pump cycle probably involves the first vibrational transition of water vapour at $6 \mu\text{m}$ wavelength. Hence, the maser will have a conversion loss (the ratio of the pump frequency to maser frequency) of about 2,000. If the pumping source is blackbody emission from the circumstellar dust in the masing cloud, then a total luminosity of $10^5 L_{\odot}$ is required. This luminosity is about the most that can be expected from a newly-formed star.

Slightly more efficient pumps have recently been described by Strelitskij¹⁰. Hence, the appearance of masers of $10^2 L_{\odot}$ requires either a collection of ~ 100 newly-formed stars within a volume of dimension 2 pc or a wholly novel form of pump source.

It is clearly important to measure the sizes of the individual maser spots and the maser cluster. Unfortunately, the best resolution that can be obtained with an interferometer spanning the Earth is ~ 200 microarc seconds, corresponding to about 10^{16} cm at a distance of 5 Mpc. Hence, resolution of extragalactic masers will have to wait until VLBI experiments can be made with an antenna on a space probe. One such experiment, named QUASAT, is in the preliminary planning stage¹¹. An international meeting sponsored by the European Space Agency and the US National Academy of Sciences was convened last month in Vienna to discuss the design and aims of the mission, which could be flown as early as 1992.

Claussen *et al.* found that all the powerful extragalactic masers are associated with galaxies having high infrared luminosity and indications of activity in their nuclei. It is commonly believed that the high infrared luminosity is due to the presence, in the nucleus, of a large number of hot massive stars that are enshrouded in dust¹². The ultraviolet radiation from these stars heats the dust, which then radiates strongly at infrared wavelengths. These stars have short lifetimes ($\sim 10^6$ yr), and their high concentration implies a burst of star formation. Such bursts may be triggered by tidal interactions with nearby galaxies. An extreme example of the star-burst phenomenon may be the IRAS source 0422+009: Aaronson and Olszewski¹³ hypothesize that 10 per cent of the mass of the galaxy is involved in the burst. These massive stars will become supernova, and a burst of star formation should be followed by the appearance of nonthermal radio and X-ray emission due to synchrotron radiation and possibly other forms of activity¹⁴. Hence, there may be a connection between star-burst galaxies and, for example, galaxies with active nuclei such as Seyfert galaxies. Strong water-vapour masers should provide a diagnostic tool for tracing the onset of a star burst.

A careful study of the kinematics of the powerful extragalactic masers should allow a direct determination of their distances from the Sun. If the maser spots are distributed randomly around one or more stars, then the root-mean-square deviation in line-of-sight velocity should equal the root-mean-square deviation in transverse velocity. The line-of-sight velocities can be measured via the Doppler effect and the transverse velocities by multiplying the angular velocities by the distance. Hence, by comparing line-of-sight velocities and transverse angular velocities, the distance can be estimated. This technique has been used to estimate the distances to galactic masers as far away as 7 kpc (ref. 5). One of

the masers detected by Claussen *et al.* had components spread over a range of 600 km s⁻¹. To determine the velocity to an accuracy of 30 km s⁻¹, for a source at 5 Mpc, the measurement accuracy must be 1 microarc second per year. Such a motion can be detected from measurements of the relative positions of maser spots over time scales of several years. Measurements of such accuracy are barely possible with current VLBI techniques, but these techniques are improving rapidly. The accuracy of the estimate of the distance using the described statistical parallax technique, for the case where the velocities are well determined, is about $DN^{-1/2}$, where D is the distance and N is the number of maser spots measured. Thus, an analysis of a maser with 100 spots should give a formal estimate of the distance to an accuracy of 10 per cent. This technique could lead to an improved

distance scale to the nearby galaxies and an improved value of the Hubble constant. □

1. Cook, A.H. *Celestial Masers* (Cambridge University Press, 1977).
2. Reid, M.J. & Moran, J.M. *Astr. Astrophys.* **19**, 231 (1981).
3. Cheung, A.C. *et al. Nature* **221**, 626 (1969).
4. Claussen, M.J., Heiligman, G.M. & Lo, K.Y. *Nature* **310**, 298 (1984).
5. Genzel, R. *et al. Astrophys. J.* **247**, 1039 (1981).
6. Churchwell, E. *et al. Astr. Astrophys.* **54**, 969 (1977).
7. Marques dos Santos, P.M. & Lepine, J.R.D. *Nature* **278**, 34 (1979).
8. Gardner, F.F. & Whiteoak, J.B. *Mon. Not. R. astr. Soc.* **201**, 13P (1982).
9. Baan, W.A. & Haschick, A.D. *Astrophys. J.* **279**, 541 (1984).
10. Strelitskij, V.S. *Mon. Not. R. astr. Soc.* **207**, 339 (1984).
11. Preston, R.A. *et al. in Very Long Baseline Interferometry Techniques*, 417 (Cepadues, Toulouse, 1984).
12. Rieke, G.H. *et al. Astrophys. J.* **238**, 24 (1980).
13. Aaronson, M. & Olszewski, E.W. *Nature* **309**, 414 (1984).
14. Weedman, D.W. *et al. Astrophys. J.* **248**, 105 (1981).

James Moran is a senior radio astronomer at the Harvard-Smithsonian Center for Astrophysics, Cambridge, Massachusetts 02138.

Plant life history

Death of the elusive biennial

from Jonathan Silvertown

FOR the purposes of analysis, ecologists have tended to classify plants as annuals, biennials or perennials. Annuals arise from seed, grow, flower and die within a year; biennials flower and die in the second year after germination; and perennials live for many years and flower many times. Annuals may increase at a yearly rate equal to the fraction of plants which survive till flowering, multiplied by their seed production. This gives an annual a strong advantage over a biennial which, living longer but, for the sake of argument, surviving just as well to flowering and producing as many seeds, will take two years to accomplish the same seed output. Assuming the same mortality and seed production, perennials are favoured over both annuals and biennials because they combine regular yearly seed production with survival of the flowering plant; the magnitude of their advantage depends on the ratio of seedling mortality in the first year of life to that of older plants¹. With such a disadvantage, why do biennials exist at all?

First, a clarification is necessary. Several vegetable crops, among them the leek, behave as biennials but all, but one², demographic studies of purported biennials have demonstrated that, outside cultivation, so-called biennials often live three, four or more years before flowering and dying. These plants are more properly described as semelparous (single-reproducing) perennials and should have even lower fitness than true biennials in normal conditions. Foxglove, once a famous 'biennial', is actually an iteroparous (multiple-reproducing) perennial in early recolonization of forest gaps, although the cohorts that arise late in succession behave rather like biennials³.

If the theoretical disadvantages of their life history are such that the paucity of biennials is understandable, the existence of semelparous perennials, which delay reproduction for longer still, is even more



FOXGLOVE

curious. Though semelparous perennials are rare in floras as a whole (only 1.4 per cent of the North American flora are semelparous perennials compared with 21.3 per cent of annuals)⁴, they are very common in some families. Thirty per cent of European umbellifer species show this behaviour. So the disadvantages of this life history cannot be as great as they seem.

Some of the disadvantage is overcome because semelparous perennials produce more seeds than annuals, as theory predicts they should. Delayed reproduction is favoured when seed production increases

disproportionately with the length of the delay. This appears to account for the semelparous perennial behaviour of fuller's teasel⁵. But are there also ecological conditions which favour them?

Semelparous perennials are particularly common in certain habitats, notably where there is intermittent disturbance of the vegetation such as treefall gaps in forests. Seedlings require a gap in which to regenerate successfully⁶ and it so happens that intermittent gaps are just the kind in which annuals lose their advantage because, without annually available space, they are unable to produce a new generation every year. In the same circumstances, however, established semelparous perennials are still able to accumulate the resources needed for seed production⁷.

Semelparous perennials are also common in the early stages of succession but they are eventually squeezed out by iteroparous perennials. Harper⁸ first pointed out that the disadvantage of delayed reproduction disappears in declining successional populations because biennials die more slowly than annuals; van der Meijden and van der Waals-Kooij⁹ have shown that even greater reproductive delays can be an advantage in a declining population. The results of stochastic simulations point in the same direction¹⁰. A recent demographic analysis shows that the 'biennial' habit of wild carrot is favoured over the annual one when successional changes in the habitat are taken into account¹¹.

Another discovery, first reported for teasel¹² and now for many semelparous perennials, is that plants must reach a minimum size before flowering and that this can largely account for the length of their reproductive delays. A life history model which compares the fitness of semelparous perennials that cue reproduction by size with those that cue it by age (as must a true biennial) has shown that cueing by size is a superior strategy in a deteriorating environment. A reduction in available resources causes a delay in reproduction for size-cueing plants, which is less damaging to fitness than the reduction in seed output that it causes in age-cueing ones¹³. All the recent studies point to the same conclusion: the biennial is dead — long live the semelparous perennial. □

1. Charnov, E.L. & Schaffer, W.M. *Am. Nat.* **107**, 791 (1973).
2. Klemow, K.M. & Raynal, D.J. *J. Ecol.* **69**, 33 (1981).
3. Baalen, J. van & Prins, E.G.M. *Oecologia* **58**, 84 (1983).
4. Hart, R. *Am. Nat.* **111**, 792 (1977).
5. Caswell, H. & Werner, P.A. *Ecology* **59**, 53 (1978).
6. Gross, K.L. *J. Ecol.* **68**, 919 (1980).
7. Silvertown, J.W. *Am. Nat.* **121**, 448 (1983).
8. Harper, J.L. *Population Biology of Plants* (Academic, New York, 1977).
9. Meijden, E. van der & Waals-Kooij, R.E. van der *J. Ecol.* **67**, 131 (1979).
10. Klinkhamer, P.G.L. & de Jong, T.J. *Ecol. Modelling* **20**, 223 (1983).
11. Lacey, E.P. *et al. Am. Nat.* **122**, 114 (1983).
12. Werner, P.A. *Oecologia* **20**, 197 (1975).
13. Hirose, T. *Bot. Mag. Tokyo* **96**, 37 (1983).

Jonathan Silvertown is in the Department of Biology, The Open University, Milton Keynes MK7 6AA.

Plant development

Antibodies to the rescue

from David Hanke

IN biology, the most dramatic advances in understanding can generally be traced back to a new technique. Our knowledge of plant development looks to be poised for just such an expansion, judging by recent papers, including one on page 321 of this issue¹, reporting results obtained using powerful immunological techniques. In the antibody we seem at last to have an experimental tool with the sensitivity and selectivity to match those of the control points in development.

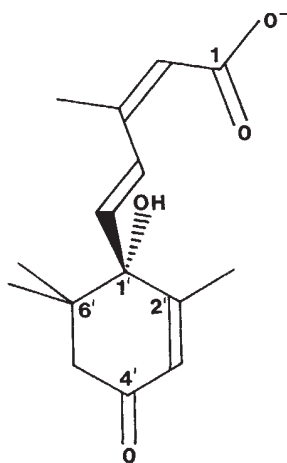
Many of these control points are sensitive to one or other of a clutch of simple organic chemicals, the plant growth substances, for which immunoassays have now been developed, most notably by Elmar Weiler². Immunoassay provides a speedy, cheap and accessible technique, harnessing the astonishing selectivity of antibodies in order to detect and identify, and the affinity in order to measure, the traces of individual growth substances in plants.

Antibodies have recently been used for the first immunocytochemical localization of a plant growth substance, dihydrozeatin, a cytokinin, in frozen sections of maize root³. A particularly ingenious immunological method was used by Jacobs and Gilbert⁴ to locate the enigmatic biochemical machinery responsible for the fixed polarity of transport of the plant hormones named auxins. They raised a large number of monoclonal antibodies to total membranes from pea stems and selected out the cell clones producing antibodies which interfered most strongly with reversible binding to the membranes by naphthylphthalamic acid, a specific non-competitive inhibitor of auxin polar transport. These monoclonal antibodies, specific for the naphthylphthalamic acid binding site, were then used to map the sites to the plasma membrane at the basal end of cells of the vascular parenchyma, exactly where the theories of polar transport had predicted they should be.

More is known about the proteins that transport growth substances across membranes than about those that mediate their developmental effects. This is because whereas transport can be measured, the immediate functions of developmental receptors cannot, because we do not know what they are. We know that such receptors must exist because analogues of the growth substances are potent only if they mimic the genuine article with a steric precision, a singular disposition of chemical groups, that only a protein would be capable of appreciating. Now, Hornberg and Weiler¹ have achieved a major methodological advance, and quite literally got to grips with potential 'receptor' proteins, by brilliantly exploiting the unique chemical structure of

another of the growth substances, abscisic acid.

Until now the search for receptors has been carried out chiefly by looking for an appropriate concentration of 'sites' in the cell which bind the growth substance reversibly and saturably and with an affinity and specificity to match the characteristics of the response of the cell to the growth substance. The number and affinity of the binding sites is determined by analysis of the relationship between the amounts of growth substance bound reversibly at dif-



Structure of *S*-abscisic acid. The asymmetric carbon atom C-1 is its chiral centre.

ferent concentrations⁵. This somewhat indirect method can generate bogus 'receptors'; for example, although only a single fusicoccin-binding protein can be isolated from plant cells, Scatchard plots of reversible fusicoccin binding to plant membranes are always biphasic⁶. Weiler and Hornberg have avoided this pitfall by covalently tagging the binding proteins with labelled growth substance. No cross-linking reagent was used, thereby greatly reducing the potential for artefacts, because abscisic acid itself can be induced by irradiation with UV light to irreversibly bind its 'receptor' through its ketone group at position C-4'. The method could hardly be more direct.

The really clever part of the experiments, however, is the 'control'. The molecule of abscisic acid is chiral. In plants it occurs as the *S*-enantiomer only, while chemically synthesized material (much cheaper) is a racemic mixture which is difficult to resolve. Weiler and his colleagues have raised antisera specific for the *R*- and *S*-forms which enable them quickly and efficiently to purify the individual enantiomers. It is quite remarkable that immunoglobulins should be able to discriminate between the enantiomers because, as can be

seen from the diagram, the molecule of abscisic acid is almost symmetrical on either side of a plane through the optical centre — the only difference between the two halves being that C-6' carries two methyl groups whereas C-2' has one and a double bond⁷. Moreover, most plant tissues cannot distinguish between the enantiomers⁸. The best discrimination reported until now is by the stomata of barley leaves⁹. A stoma is an epidermal pore which is opened and closed by the expansion and contraction of two specialized cells which flank it, the guard cells. The responses of guard cells to growth substances are completely reversible and have to do with physiology rather than growth and development, but, rather like the thyroid and steroid hormones of animals, plant growth substances can affect many diverse processes. Thus an increase in the concentration of abscisic acid signals lack of water and both development and physiology may be adjusted accordingly.

Working with broad bean leaves, Weiler and Hornberg find that *R*-abscisic acid has no effect on stomatal aperture over the first 30 minutes. This compound, which is virtually identical to the natural active form except in its chirality, is therefore a superb control. In the search for receptors only those guard-cell proteins that bind *S*-abscisic acid and not *R*-abscisic acid need be considered. In fact, Weiler and Hornberg find a large excess of cross-linking to proteins of guard-cell protoplasts by *S*- as opposed to *R*-abscisic acid. Similarities between the characteristics of the binding and the characteristics of the physiological response amount to an impressive correlation entirely consistent with the hypothesis that these binding proteins, anything up to three it seems, are involved in the response.

Because proteolysed but intact protoplasts bind virtually no abscisic acid, Weiler and Hornberg argue that all the binding sites, which presumably include the receptors, are located on the outside of the plasma membrane. This is reasonable provided none of the proteins concerned is responsible for transporting *S*-abscisic acid across the plasma membrane¹¹ and therefore also responsible for access to internal receptors. According to the only study so far of abscisic acid transport into guard cells, in this case of the unrelated wild flower *Lamb's Lettuce*, there does not seem to be a carrier-mediated component to uptake. Moreover, the growth substance is no more effective at pH 5, when it enters the cells, than at pH 8 when, as the anion, uptake cannot be detected¹¹. It appears, therefore, that the chain of events triggered by abscisic acid in guard cells begins with receptor binding at the plasma membrane.

What little (circumstantial) evidence there is for the location of receptors for auxin has favoured binding sites on the endoplasmic reticulum^{12,13}, so receptors for different growth regulators may well function quite differently. Even for the same

growth regulator there may be different receptors in different tissues — most tissues do not distinguish between *R*- and *S*-abscisic acid for instance. Even for the same tissue the receptors for the same growth substance may vary from species to species. Thus broad bean guard cells are completely insensitive to the *S*-abscisic acid metabolite, phaseic acid; whereas for guard cells of *Commelina communis* phaseic acid is just as effective as abscisic acid; and other plants, such as cocklebur, have guard cells which respond to phaseic acid but less sensitively than they respond to abscisic acid¹⁴. It seems likely that there is a rich and elaborate world of growth substance receptors. Immunological

techniques can provide the selectivity and sensitivity needed to explore it. □

1. Hornberg, C. & Weiler, E.W. *Nature* **310**, 321 (1984).
2. Weiler, E.W. *Physiol. Pl.* **54**, 230 (1982).
3. Zavala, M.E. & Brandon, D.L. *J. Cell Biol.* **97**, 1235 (1983).
4. Jacobs, M. & Gilbert, S.F. *Science* **220**, 1297 (1983).
5. Rubery, P.H. *A. Rev. Pl. Physiol.* **32**, 569 (1981).
6. Aducci, P. *et al.* in *Plant Growth Substances 1982* (ed. Wareing, P.F.) 395 (Academic, London, 1980).
7. Milborrow, B.V. *A. Rev. Pl. Physiol.* **25**, 259 (1974).
8. Sondheimer, E. *et al.* *Science* **174**, 829 (1971).
9. Cumming, W.R. & Sondheimer, E. *Planta* **111**, 365 (1973).
10. Astle, M.C. & Rubery, P.H. *Planta* **157**, 53 (1983).
11. Hartung, W. *Pl. Cell Environ.* **6**, 427 (1983).
12. Walton, J.D. & Ray, P.M. *Pl. Physiol.* **68**, 1334 (1981).
13. Stoddart, J.L. & Venis, M.A. *Enc. Pl. Physiol. N.S.* **9**, 462 (1980).
14. Sharkey, T.D. & Raschke, K. *Pl. Physiol.* **65**, 291 (1980).

David Hanke is in the Department of Botany, University of Cambridge, Downing Street, Cambridge CB5 8BL.

Virology

Tissue-specific transformation by human T-cell leukaemia virus

from Robin Weiss

ONE of the ironies of modern virology is that our knowledge of molecular structure far outstrips our understanding of pathogenesis. In the case of human T-cell leukaemia virus type 1 (HTLV-1), the nucleotide sequence of the entire genome was published one year ago by Yoshida and his colleagues in Tokyo¹ at a time when next to nothing was known about its means of natural transmission or mechanism of oncogenesis. Yoshida's group did, however, reveal an unexpected sequence, the *X* gene, located between the *env* gene and the 3' long terminal repeat (LTR; Fig. 1c). In two papers in the current issue of *Science*^{2,3}, Haseltine *et al.* analyse the *X* region of human T-cell leukaemia virus type 2 (HTLV-2) and propose that a protein encoded by the 3' portion of *X* may act as a *trans*-acting transcriptional activator of the LTR and cellular control elements. The papers may provide an important clue to the way in which HTLV transforms cells.

Earlier models of transformation by oncogenic retroviruses are based either on the transduction of oncogenes (Fig. 1a) or on the chromosomal insertion of the provirus adjacent to a cellular proto-oncogene (Fig. 1b), thereby enhancing its expression^{4,5}. HTLV-1 does not fit either model, as the viral genome neither contains an oncogene of host origin, nor integrates in the same chromosomal domain in different clonal tumours. Hahn *et al.*⁶ had reported a common integration site in 3 out of 11 lymphoma lines but this claim was subsequently retracted⁷, and a recent survey of 35 lymphomas by Seiki *et al.*⁸ did not reveal a common locus. Bovine leukosis virus, which is distantly related to the HTLV family, also induces lymphomas with widely dispersed integration sites^{9,10}, and this virus has now been found to possess an *X* gene¹¹ with similar open reading frames to HTLV-1.

Like Epstein-Barr virus, HTLV is able to transform normal cells in culture to a state of unlimited proliferation potential. Since this phenomenon of 'immortalization' by HTLV was first demonstrated by Miyoshi *et al.*¹², both HTLV-1 and HTLV-2 have been widely used to obtain clones of immortal T cells¹³⁻¹⁵. Transformation appears to be restricted to T cells, usually with an OKT4⁺ phenotype, like that of tumours induced *in vivo*. B cells^{15,16} and some non-lymphoid lines¹⁷ can also be infected with HTLV, but OKT4⁺ T cells appear to be the most permissive cells for both viral replication and transformation.

The molecular basis of T-cell tropism has now been investigated by Golde and Haseltine and their co-workers. Both groups used recombinant plasmids to study the influence of sequences within the LTR on gene expression in animal cells of dif-

ferent types. Chen *et al.*¹⁸ made constructs of the HTLV-2 LTR with the selectable gene, *neo*^R, while Sodroski *et al.*³ used recombinants of the LTR and the chloramphenicol acetyltransferase (*CAT*) gene. Chen *et al.* find that *neo*^R expression is restricted to lymphoid cells and conclude that expression is governed by *cis*-acting functions of the HTLV-2 LTR. They suggest that *trans*-acting regulatory proteins expressed only in lymphoid cells may act directly on the LTR to enhance expression, or alternatively, specific proteins may suppress LTR function in non-lymphoid cells.

Sodroski *et al.*³ have taken their experiments further and suggest that a product of the HTLV *X* gene itself acts as the *trans*-regulatory protein on LTR function in the transient *CAT* expression system. This conclusion is based on observations made using non-lymphoid human osteosarcoma (HOS) cells, which provide a system in which the same line can be studied before and after infection with HTLV-1 (ref. 17). *CAT* expression from HTLV-1 LTR-*CAT* recombinants is 100-fold greater in HTLV-1-infected HOS cells than in control HOS cells, but expression of HTLV-2 LTR-*CAT* recombinants is not detectable in either cell type. Sodroski *et al.* also observe that HTLV-1 LTR-*CAT* expression is much more efficient in T cells pre-infected and transformed by HTLV-1 or HTLV-2 than in uninfected B- or T-cell lines, although the high expression of the HTLV-2 LTR-*CAT* recombinant is restricted to HTLV-2-transformed cells. Unfortunately, a cloned T-cell line uninfected and infected by HTLV was not available for comparative studies such as were performed with HOS cells.

One HTLV-1-immortalized line, C81/66, expresses only the *X* gene, yet it is highly permissive for LTR-*CAT*; hence the hypothesis that the *X* product acts in *trans* on the LTR³. Thus the expression of a proviral *X* gene appears to enhance expression

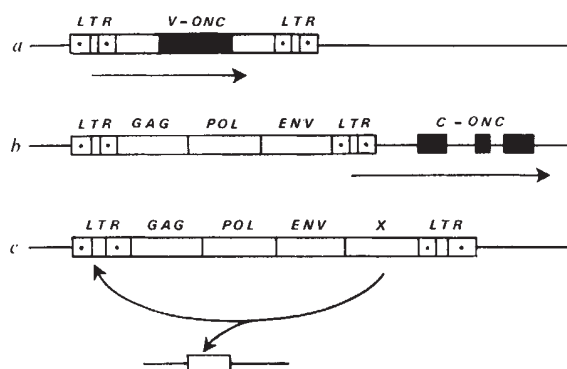


Fig. 1 Molecular models of retrovirus oncogenesis. The proviral DNA is shown flanked by 5' and 3' long terminal repeats (LTRs) integrated into host cellular DNA. *a*, LTR-initiated transcription of a transduced oncogene (*v-onc*) within a defective viral genome. *b*, Integration of a non-defective provirus in the vicinity of a cellular proto-oncogene (*c-onc*) leads to its activation via enhancer or promoter sequences in the adjacent LTR. *c*, The HTLV genome carries an extra *X* gene whose product, acting in positive feedback, enhances transcription of its own LTR and also of other transcription units. The transcription of *v-onc* and *c-onc* genes in *a* and *b* is *cis*-controlled, whereas activation of the LTR or cellular genes by the *X* product in *c* is *trans*. Model *b* requires site-specific integration of the provirus, while models *a* and *c* are independent of host chromosomal locus.

of LTR-CAT plasmids. The HTLV-2 *X* product will act on HTLV-2 and HTLV-1 LTRs, whereas the *X* product of HTLV-1 does not act on the HTLV-2 LTR. The promoter of HTLV-2 LTR is probably weaker than that of HTLV-1; this may explain its more restricted tropism to T cells, as only lymphoid cells will allow sufficient initial expression for the *X* gene product to act as a positive feedback. The long open reading frame in *X* is highly conserved between HTLV-1 and HTLV-2 (ref. 2).

The notion of *trans*-acting transcriptional activation may then be extrapolated to suggest that cellular control elements similar to LTRs might also be enhanced by the *X* product. Interestingly, Holbrook *et al.*¹⁹ have just reported that a DNA sequence 5' to the human interleukin 2 (IL-2) gene shows limited regions of homology to the LTR of HTLV-1. Since HTLV-transformed cells invariably express IL-2 receptors, activation of IL-2 by HTLV could lead to an autocrine stimulation of proliferation, possibly explaining the immortalization effect. But such a neat story is dashed by another recent paper²⁰ showing that the IL-2 gene is not transcribed in HTLV-transformed cells. Perhaps expression of the IL-2 receptor gene itself is enhanced by the HTLV *X* product.

The new observations on the apparent

trans-acting regulation of HTLV expression will stimulate further exploitation of retroviruses and recombinant plasmids derived from them as probes for tissue-specific gene expression. It will be interesting to see whether the AIDS retrovirus isolates^{21,22}, which destroy rather than immortalize OKT4⁺ cells, also have related *X* genes and LTR enhancer sequences. □

1. Seiki, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 3618 (1983).
2. Haseltine, W.A. *et al. Science* **225**, 419 (1984).
3. Sodroski, J.G., Rosen, C.A. & Haseltine, W.A. *Science* **225**, 381 (1984).
4. Hayward, W.S., Neel, B.G. & Astrin, S.M. *Nature* **290**, 475 (1981).
5. Payne, G.S., Bishop, J.M. & Varmus, H.E. *Nature* **295**, 209 (1982).
6. Hahn, B. *et al. Nature* **303**, 253 (1983).
7. Hahn, B. *et al. Nature* **305**, 340 (1983).
8. Seiki, M. *et al. Nature* **309**, 640 (1984).
9. Onuma, M. *et al. Microbiol. Immun.* **26**, 813 (1982).
10. Kettman, R. *et al. J. Virol.* **47**, 146 (1983).
11. Rice, N. *et al. Personal communication.*
12. Miyoshi, I. *et al. Nature* **294**, 770 (1981).
13. Yamamoto, N. *et al. Science* **217**, 737 (1982).
14. Popovic, M. *et al. Science* **219**, 856 (1983).
15. Chen, I., Quan, S.G. & Golde, D.W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7006 (1983).
16. Yamamoto, N. *et al. Nature* **299**, 367 (1982).
17. Clapham, P. *et al. Science* **222**, 1125 (1983).
18. Chen, I., McLaughlin, J. & Golde, D.W. *Nature* **309**, 276 (1984).
19. Holbrook, N.J., Leiber, M. & Crabtree, G.R. *Nucleic Acids Res.* **12**, 5005 (1984).
20. Arya, S. *et al. Science* **223**, 1086 (1984).
21. Barré-Sinoussi, F. *et al. Science* **220**, 868 (1983).
22. Gallo, R.C. *et al. Science* **224**, 500 (1984).

Robin Weiss is at the Institute of Cancer Research, Fulham Road, London SW3 6JB.

bulence so difficult to measure.

Evidence for the importance of wave-induced turbulence in the ocean surface layers is fragmentary and at the edge of confidence in experimental results. The most direct and convincing evidence comes from recent measurements of turbulence made from an instrumented tower in Lake Ontario. Kitaigorodski *et al.*⁵ found that the thickness of a surface layer in which wave-generated turbulence dominated that produced by other sources was of order ten times the root-mean-square wave amplitude. Indirect measurements based on the vertical distribution of bubbles below the surface⁶ support this result. What is astonishing is that such a thick layer should, on average, be affected, as wave-breaking is an intermittent and, when measured at a fixed position, rather infrequent event. It may be that another process ascribed to the action of surface waves is responsible for the downward advection of turbulence from the surface. In strong winds, parallel bands of foam or other flotsam aligned in the wind direction, and known as wind rows, appear on the sea surface. These are lines of convergence in alternating vortices known as Langmuir circulation⁷ (after the man who first investigated the phenomena) and recent measurements show that the downward velocities beneath the wind rows may be several centimetres per second, sufficient to distort the distribution of turbulence and perhaps to thicken the layer appreciably.

Further surprises may be in store. Studies of drift currents⁸ show that the turbulent roughness of the surface is some two orders of magnitude greater than expected from atmospheric analogues. On this basis surface waves in moderate winds have a roughness scale for the water almost equal to that of a forest canopy for the air above.

Shay and Gregg have not measured the surface layer where wave-induced turbulence may dominate, but results may soon be available from a profiling instrument specially designed to profile upwards from depth to the surface in the wave zone⁹. We may confidently expect that, within the next few years, some of the intricate and fascinating connections between dynamical processes in the upper-ocean boundary layer will be unravelled so that models can be developed with more realistic representations of both local atmospheric forcing and waves. □

1. Shay, T.J. & Gregg, M.C. *Nature* **310**, 282 (1984).
2. Jones, I.S. & Kenney, B.C. *J. geophys. Res.* **82**, 1392 (1977).
3. Dillon, T.M. *et al. Nature* **290**, 390 (1981).
4. Hunt, J.C.R. *J. Fluid Mech.* **138**, 161 (1984).
5. Kitaigorodski, S.A., Donelan, M.A., Lumley, J.L. & Terray, E.A. *J. phys. Oceanogr.* **13**, 1988 (1983).
6. Thorpe, S.A. *J. phys. Oceanogr.* (in the press).
7. Leibovich, S. A. *Rev. Fluid Mech.* **15**, 391 (1983).
8. Churchill, J.H. & Csanady, G.T. *J. phys. Oceanogr.* **13**, 1669 (1983).
9. Park, M.M. *et al. WAZP Observations during MILDEX Oct-Nov 1983* (Sch. of Oceanogr., Oregon State Univ., 1984).

S.A. Thorpe is in the Institute of Oceanographic Sciences, Godalming, Surrey GU8 5UB.

Oceanography

Probing beneath the waves

from S.A. Thorpe

A remarkable technical feat is described on page 282 of this issue of *Nature*. Because of the violence of the surface waves, precise measurement of the turbulence in the upper few tens of metres of the ocean is a daunting task. It demands great skill as well as stable instruments capable of high resolution, and few laboratories are adequately equipped. Nevertheless, Shay and Gregg at the University of Washington have now succeeded in using data from falling probes to show that in a convective layer below the surface of the ocean, a key property of the turbulence — the rate of dissipation of turbulence kinetic energy — scales in the same way as it does in the convective boundary layer of the atmosphere¹. This is not altogether a surprise. Others have suggested, and searched for, similarities between the ocean and atmospheric boundary layers^{2,3}. It is, however, as well to be sure and to test the empirical relationships (empirical at present, but recently there have been theoretical advances⁴). Shay and Gregg provide a sounder basis for models which incorporate well-established information from the atmosphere. Their measurements also have practical implications, for example for estimating the distribution of nutrients which support phytoplankton, and for predicting the extreme currents

which offshore structures must be able to withstand.

But is the upper ocean really like the lower atmosphere? What about the waves? There are strong indications that surface waves contribute significantly to ocean turbulence, at least close to the surface, probably through their breaking. The precise mechanism by which they do so is not known, although possible contributory factors include the generation of vorticity when the water surface becomes multiply-connected in breaking waves, the detachment of the thin viscous boundary layer at the surface and motions in the wake of large rising bubbles of air engulfed by the breaking waves. There are basic asymmetries both in wave breaking and in the flows either side of the ocean-atmosphere interface. Thus, waves break at their crests, rather than at their troughs, and the falling wedge of water in a plunging breaker traps a bubble of air — an asymmetrical pattern; and the dynamically important level at which the mean fluid motion equals the speed of the dominant waves is located in the atmosphere rather than in the ocean. Therefore, whereas the non-wave-induced fluctuations dominate in the atmospheric surface layer, they are greatly exceeded by the wave-induced motions in the ocean surface layer. It is this which makes ocean tur-

Muscle contraction

Two states of attachment

from Maxine Clarke

ATTEMPTS to discover exactly how muscle contracts have been consistently unsuccessful because of the insufficient resolution of the available structural methods. Insect flight muscle offers the best prospects for solving the problem, and, specifically, for investigating the behaviour of the myosin crossbridges which are intimately involved in the contraction process. An article¹ in this issue of *Nature* proposes a new view of crossbridge structure based on observations of insect muscle.

Muscle contraction is brought about by the sliding of thick filaments of myosin between interdigitating thin filaments of actin. A close look at the filaments under the electron microscope reveals the presence of crossbridges (myosin 'heads') extending from the thick filament backbone across to the actin filaments. When muscle contracts, the crossbridges with bound products of ATP hydrolysis are thought to attach to actin monomers in the thin filaments at a preferred angle (often called the '90° state'). On attachment to actin the hydrolysis products are displaced, which causes a change in orientation of the attached head to an angle of about 45° with respect to the filament axis and the filaments to be pulled past each other. Binding of a new molecule of ATP then loosens the attachment of the crossbridge and the cycle can repeat^{2,3}. This basic model of contraction (see the figure) has undergone a series of refinements over the past ten years but the concept of rocking or bending of attached crossbridges as the basis of a 'power stroke' responsible for filament sliding has remained^{4,5}.

Until now, however, a crucial part of the model — the existence of more than one conformations of attached crossbridges — has lacked experimental support; only one attached crossbridge state, rigor, has ever been clearly identified despite many attempts to find other states. This rigor state, a stable equilibrium condition with no ATP or ADP bound to the crossbridges, is thought to represent the end of the power stroke. Muscle fibres in rigor have almost all the available crossbridges attached to actin, as judged by their high mechanical stiffness. A high-resolution view of the rigor crossbridge has been obtained by decorating isolated actin filaments with stoichiometric quantities of single myosin heads (also named subfragment 1, S1). An arrowhead structure is seen in which all the S1s are bound at a fixed angle of about 50° (refs 6 and 7). The structure of rigor crossbridges in intact muscle fibres has been most successfully investigated using insect flight muscle where the helical repeats of

the thick and thin filaments are identical⁸. Electron-microscope studies of rigor muscle in longitudinal section reveal crossbridges as 'double chevrons', angled structures linking the thick with the thin filaments⁹. X-ray diffraction patterns of the fibres have been interpreted in terms of crossbridges uniformly oriented at about 60° to the filament backbone¹⁰.

Taylor *et al.*¹ have now attempted a more sophisticated approach to the electron-microscope study of such muscle. They cut very thin sections of material comprising just one layer of thick and thin filaments. These two-dimensional arrays, which they name 'myac layers', were tilted in the microscope and the resultant series of views of the specimen reconstructed to give a three-dimensional image of the crossbridges in the filament lattice. The results are surprising. Each double chevron, which had been presumed to contain two pairs of crossbridges, is clearly resolved into two elements. The 'lead' chevron of each pair is shorter by 1.5 nm, is tilted at about 50° to the thin filament and probably consists of two myosin heads. The 'rear' chevron is longer (17.5 nm), is tilted at about 80° to the filament axis and probably consists of only one myosin head. Thus there seem to be conformationally distinct populations of attached crossbridges in the rigor state. Another novel feature to emerge from the reconstruction is a local variation in helical pitch of the actin, suggesting flexibility within the thin filament.

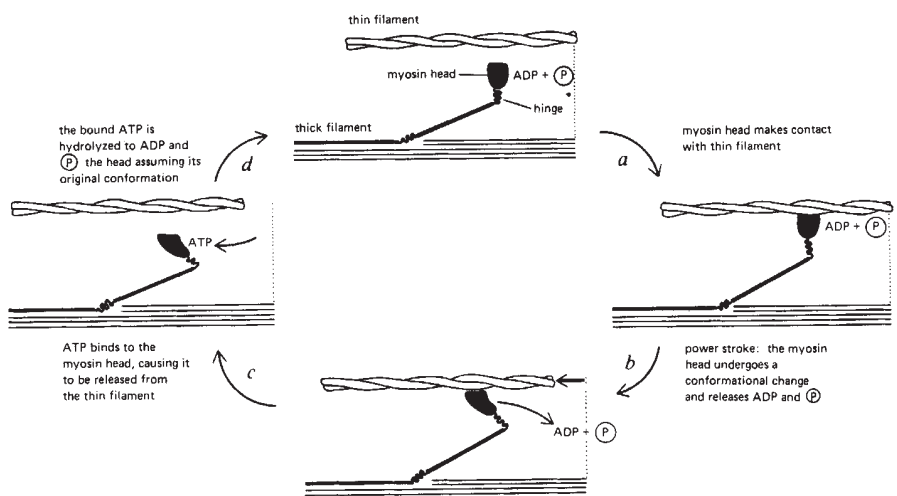
Why have these structures not been observed using other techniques? In modelling of X-ray diffraction patterns, the reason is that so far only the case of a single rigor crossbridge conformation has been taken into account¹⁰. In decorated

actin, the S1 angle is consistent with that of the lead chevron; this angle is considered to be the preferred rigor orientation¹. Because of the geometrical constraints on its attachment to the filament backbone, the rear chevron may be forced to take up a second, energetically less favourable orientation. In experiments using spin-label probes rigidly attached to a specific region of S1, only one rigor configuration is detected¹¹. Taylor *et al.* note that the rear and lead chevrons have the same angle within 6–8 nm of the actin, so if the spin label were bound to this region of the S1 it would not discriminate between the two orientations; the difference in structure of the rear and lead chevrons is in the part of the crossbridges furthest from the actin.

Some questions about the molecular organization of the myac layer remain unanswered owing to limitations of the method. Individual myosin heads are not resolved as separate structures. The exceptionally large troponin complex of insect flight muscle¹² is also unresolved, even though it is as large as a lead chevron. Nevertheless, the evidence for two types of crossbridge position, at least in rigor, is very persuasive and it raises the exciting possibility that distinct structures representing the beginning and end of the power stroke have been resolved. The next questions to be addressed are whether these two structures occur in contracting muscle and whether the transition between them is responsible for force generation. □

1. Taylor, K.A., Reedy, M.C., Cordova, L. & Reedy, M.K. *Nature* **310**, 285 (1984).
2. Lyman, R.W. & Taylor, E.W. *Biochemistry* **10**, 4617 (1971).
3. Eisenberg, E. & Greene, L.E. *A. Rev. Physiol.* **42**, 293 (1980).
4. Huxley, H.E. *Science* **164**, 1356 (1969).
5. Huxley, A.F. *J. Physiol., Lond.* **243**, 1 (1974).
6. Taylor, K.A. & Amos, L.A. *J. molec. Biol.* **147**, 297 (1981).
7. Holmes, K.C. *et al. Ultramicroscopy* **9**, 37 (1982).
8. Wray, J.S. *Nature* **280**, 325 (1979).
9. Reedy, M.K. *et al. Nature* **207**, 1276 (1965).
10. Holmes, K.C. *et al. Proc. R. Soc. B207*, 13 (1980).
11. Thomas, D.D. *et al. Biophys. J.* **33**, 21a (1980).
12. Bullard, B. *J. Muscle Res. Cell Motil.* **5**, 196 (1984).

Maxine Clarke is in the MRC Cell Biophysics Unit, 26–29 Drury Lane, London WC2B 5RL.



Model of muscle contraction: how myosin induces filament sliding. From *Molecular Biology of the Cell* by B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts and J.D. Watson (Garland, 1983).

Palaeontology

Selective extinctions and terminal Cretaceous events

from Eric Buffetaut

THE idea that mass extinctions have been caused by impacts of extraterrestrial bodies on the Earth, as suggested in 1980 by Alvarez *et al.*¹, seems to be gaining ground, particularly in the aftermath of possible evidence of a periodic pattern to the extinctions (see ref. 2). However, the processes by which large-scale but selective extinctions can be triggered by such events are not yet fully understood, and many palaeontologists, in particular, still favour a more gradual process of extinction². In the continuing debate between catastrophists and gradualists, which has centered on terminal Cretaceous extinctions, the focus has been on the animals and plants that became extinct, and little attention has been paid to the numerous and varied groups that survived. Since most of these groups are still represented today, we know much more about the physiological and ecological requirements of these 'survivors' than about those of, for example, dinosaurs or ammonites. A confrontation of this kind of data with some current hypotheses about terminal Cretaceous extinctions casts serious doubts on some of the environmental changes postulated to have occurred at the end of the Cretaceous.

As reconstructed by authors such as Hsü³ and Alvarez⁴, the postulated effects of asteroid or comet impacts are so devastating that one may wonder why the available fossil record suggests that many groups of organisms were virtually unaffected by whatever happened at the end of the Cretaceous. Among the hypothetical consequences of catastrophic impacts are drastic and rather sudden temperature changes, but it is difficult to reconcile such proposals with the fossil record of non-dinosaurian reptiles. The case of crocodilians is especially revealing in this respect. These animals are known to be very sensitive to temperature changes, and it is extremely unlikely that they could have survived the considerable changes in temperature suggested by some catastrophists and, yet, fossil evidence shows that crocodilians were not noticeably affected by terminal Cretaceous events⁵. The *ad hoc* explanations put forward to account for their survival (Hsü, for example, has suggested that their eggs survived by being buried in mud³) are, to say the least, unconvincing (dinosaur eggs would have been preserved, too).

Gradualist hypotheses are no better at explaining the survival of crocodilians and other reptiles but not dinosaurs at the end of the Cretaceous. The hypotheses usually imply that the Cretaceous extinctions were the result of worldwide climatic change,

and possibly marine regression^{2,6}. But since the decline of crocodilians in many parts of the world during the Tertiary seems to have been linked to progressive climatic deterioration^{7,8}, it is difficult to see how crocodilians could have survived even more extensive changes at the end of the Cretaceous.

Terminal Cretaceous extinctions were selective. Most of the land vertebrates which survived were small forms, and freshwater communities were less affected than purely terrestrial ones⁹. At first sight, this is difficult to explain within the framework of either catastrophist or gradualist hypotheses. However, an analysis in simple trophic terms may be revealing. Apparently, on the continents, terminal Cretaceous extinctions mainly affected large terrestrial herbivores and the predators which fed on them. Almost all of them were dinosaurs. This suggests that the extinctions were confined to the members of a food chain that began with land plants, which were the first to be stricken. This would explain why freshwater vertebrates, which are not directly dependent on land plants for their food, were unaffected. Small land vertebrates, such as squamates or mammals, which were mainly carnivorous, insectivorous or omnivorous, also did not need large amounts of fresh plant material to survive, and would have suffered relatively little if the 'vegetation crisis' did not last too long. There are conflicting opinions as to whether the fossil record indicates large-scale extinctions in the plant kingdom at the end of the

Cretaceous but detailed analysis of a North American locality by Orth *et al.*¹⁰ has revealed a sharp drop in angiosperm pollen abundance at the Cretaceous-Tertiary boundary. This suggests a brief crisis in the plant kingdom, followed by recuperation, perhaps aided by surviving seeds.

Assuming there was a brief crisis in the plant kingdom, what could have caused it? An attractive possibility is the introduction of a large amount of dust into the atmosphere because that could have inhibited photosynthesis. The cause of the dust is a matter of debate. It could have been the passage of the Earth through an interstellar cloud¹¹, volcanic eruption¹² or the impact of an asteroid.

Hence, impact hypotheses need not be rejected but the more drastic hypothetical consequences of an impact are not supported by the survival of some reptiles and are not needed to explain the extinction of the dinosaurs. On the whole, even though it may seem paradoxical, the selective character of the terminal Cretaceous extinctions suggests that they were caused by an event that was not very brutal or long-lasting but possibly rather sudden. If this interpretation is correct, the Mesozoic world may have ended neither with a bang, nor with a whimper, but with something in between. □

1. Alvarez, W. *et al. Science* **208**, 1095 (1980).
2. Hallam, A. *Nature* **308**, 686 (1984).
3. Hsü, K.J. *Nature* **285**, 201 (1980).
4. Alvarez, W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 627 (1983).
5. Buffetaut, E. *Mem. Soc. geol. Fr.* **139**, 47 (1980).
6. Ginsburg, L. *C. r. hebd. Séanc. Acad. Sci., Paris* **298** (11), 317 (1984).
7. Berg, X. *Geol. Rdsch.* **54**, 328 (1964).
8. Buffetaut, E. *9e Réunion ann. des Sciences de la Terre*, 101 (1982).
9. Van Valen, L. & Sloan, R.E. *Evol. Theory* **2**, 37 (1977).
10. Orth, C.J. *et al. Science* **214**, 1341 (1981).
11. Renard, M. & Rocchia, R. *Recherche* **153**, 393 (1984).
12. Zoller, W.H. *et al. Science* **222**, 1118 (1983).

Eric Buffetaut works as a palaeontologist for the Centre National de la Recherche Scientifique at the Laboratoire de Paléontologie des Vertébrés, Université Paris VI, 4 place Jussieu, 75230 Paris Cedex 05.



100 years ago

A RECENT writer in the *North China Herald* discusses the part played by mercury in the alchemy and *materia medica* of the Chinese. Cinnabar was known to them in the seventh century before the Christian era, and its occurrence on the surface of the earth was said to indicate gold beneath. Their views on the transformation of metals into ores and ores into metals by heat and other means took the form of a chemical doctrine about a century before Christ, and there is now no reasonable doubt that the Arabian Geber and others (as stated by Dr. Gladstone in his inaugural address to the Chemical Society) derived their ideas on the transmutation of metals into gold and the belief in immunity from death by the use of the

philosopher's stone from China. Among all the metals with which the alchemist worked, mercury was pre-eminent, and this is stated to be really the philosopher's stone, of which Geber, Kalid, and others spoke in the times of the early Caliphs.

In China it was employed excessively as a medicine. On nights when dew was falling, a sufficient amount was collected to mix with the powder of cinnabar, and this was taken habitually till it led to serious disturbance of the bodily functions. In the ninth century an Emperor, and in the tenth a Prime Minister, died from overdoses of mercury. Chinese medical books say it takes two hundred years to produce cinnabar; in three hundred years it becomes lead; in two hundred years more it becomes silver, and then by obtaining a transforming substance called 'vapour of harmony' it becomes gold. This doctrine of the transformation of mercury into other metals is 2000 years old in China. The Chinese hold that it not only prolongs life, but expels bad vapours, poison, and the gloom of an uneasy mind.

From *Nature* **30**, 299, 24 July 1884.

Modern dynamics and classical analysis

Michael Tabor

Department of Applied Physics and Nuclear Engineering, Columbia University, New York, New York 10027, USA

Nonlinear effects in ordinary and partial differential equations can result in a wide variety of phenomena ranging from strange attractors to solitons. Despite their great diversity, many nonlinear equations have certain fundamental properties in common which are revealed by studying the types of singularities their solutions can exhibit. Many of the mathematical tools required for this study can be found in the classical literature.

NONLINEAR ordinary and partial differential equations model an enormous variety of natural processes ranging from the simple swing of a pendulum to the complicated turbulent flow of a fluid. Despite the great diversity of their mathematical forms and the processes that they describe, many of these equations have certain fundamental properties in common. Furthermore, certain mathematical techniques—many of them from classical analysis—can be used to study these equations and provide a more unified framework to understand their properties.

One's introduction to nonlinear ordinary differential equations is usually through classical mechanics¹. Here, for example, one learns how to solve the equations of motion for a pendulum. For very small displacements, the equations are linear and the solution is immediately written in terms of the sine and cosine functions. For larger displacements the equations are nonlinear but here again an 'exact' solution can be found in terms of the so-called 'elliptic' functions. Other mechanical systems such as simple configurations of oscillators (with or without friction), certain types of spinning top or the Kepler model of planetary orbits (the 'two-body' problem) may all be solved exactly and their solutions found to have, to varying degrees of complexity, nice periodic behaviour. Such systems are termed 'integrable'. Slightly more complicated, and usually physically more relevant, mechanical systems, such as configurations of nonlinearly coupled oscillators or the 'three-body' problem of celestial mechanics (the Earth's orbit about the Sun perturbed by Jupiter), do not seem to have 'exact' solutions. These are the 'nonintegrable' systems of classical mechanics and they constitute the generic (typical) class of mechanical systems (Fig. 1). Attempts to solve the three-body problem became a major preoccupation of many mathematicians of the nineteenth and early twentieth centuries². The major difficulty was that no known forms of perturbation theory expansions would converge. Thus, not only was no 'exact' solution possible but no statements about the long-time stability of solutions were possible either.

The inability to make progress with this problem was neatly summarized by Max Born, who was attempting (unsuccessfully) to find the classical orbits of the electrons in the helium atom³. He wrote "it would indeed be remarkable if Nature fortified herself against further advance in knowledge behind the analytical difficulties of the many-body problem". In Born's case the problem was solved with the advent of quantum mechanics—but the classical problem remained.

Recently, however, our understanding of this problem has increased through the proving of certain important theorems and the advent of high speed computers. The theorems (due to Kolmogorov, Arnold and Moser) are concerned with the effect of nonintegrable perturbations on integrable systems and explain when the traditional perturbation theories diverge⁴. The computers have enabled us to study numerically the solutions of nonintegrable systems and reveal a generic phase space structure of remarkable complexity. The solutions to systems of nonintegrable differential equations are very sensitive to initial conditions. Some solutions still evolve in a smooth periodic manner whereas others evolve in a highly chaotic, although absolutely deterministic manner, progressing in time (and space) in an apparently random way. The study of 'deterministic chaos' in a variety of model systems has become an active area of research with the promise of useful applications to important physical processes⁵.

Despite the current activity in nonlinear dynamics, a fundamental question remains. Given a system of equations, how can one tell *a priori* whether or not they are integrable? To date, this identification, which requires the finding of the 'integrals of motion', has proceeded by means of analytical ingenuity, luck or computer experiment. The latter course, although valuable, can never be conclusive.

The answer to this problem probably lies in the classic work of the Russian mathematician Sofya Kovalevskaya on the problem of the rotation of a rigid body (a top) about a fixed point.

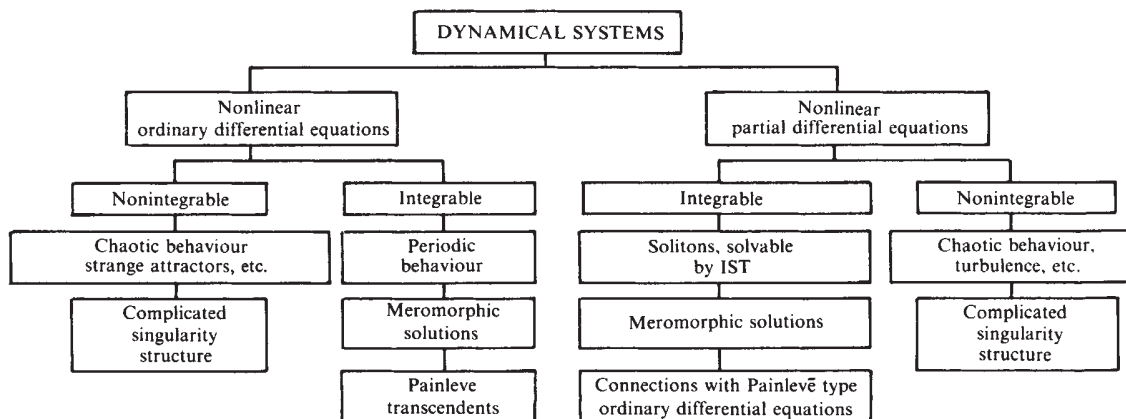


Fig. 1 Scheme showing subdivisions of the dynamical systems.

Kovalevskaya

Before describing her scientific work, we should outline Kovalevskaya's life story⁶. She was born in 1850; the middle child of a retired Russian general. At that time, the pursuit of knowledge was not considered a suitable activity for young ladies and this regime was rigorously enforced by an acerbic English governess. Nonetheless, Kovalevskaya was able to indulge in some clandestine reading of scientific and mathematical texts borrowed from an uncle's library. Once it was appreciated that she was something of a prodigy her father allowed her to undertake more formal studies with a private tutor. However, in those days the only respectable way a young lady could leave home (and hence continue her studies at university) was to get married. Such a marriage was arranged with this end in mind and she and her student husband set out for Germany where they both hoped to pursue their studies. Unfortunately, it soon became apparent that as a woman she was barred from attending any university in Germany, let alone anywhere else in Europe. However, Weierstrass, recognizing her talent, gave her private lessons. Obtaining a doctorate presented many problems not least of which was that women were barred from attending (let alone participating in) the required public thesis defence. To overcome this obstacle she wrote three dissertations which Weierstrass 'hawked' around various German campuses until the University of Gottingen finally agreed to award her a doctorate without having to make a thesis defence. This was in fact, just the beginning of an outstanding scientific career. Among other things she was awarded the Bordin prize of the Paris Academy of Science in 1888 for her work on the rigid body problem—her work was considered so outstanding that the prize money was trebled. She finally became a professor at the University of Stockholm, being one of the first women in Europe to hold a senior university faculty position. She died of pneumonia aged 41.

Her classic work on the rigid-body problem concerned the solution of the Euler–Poisson equations which describe the motion of a top spinning about a fixed point⁷. They are a set of six, first-order, nonlinearly-coupled, ordinary differential equations of the form

$$\begin{aligned} A \frac{dp}{dt} &= (B - C)qr - \beta z_0 + \gamma y_0 & \frac{d\alpha}{dt} &= \beta r - \gamma q \\ B \frac{dq}{dt} &= (C - A)pr - \gamma x_0 + \alpha z_0 & \frac{d\beta}{dt} &= \gamma p - \alpha r \\ C \frac{dr}{dt} &= (A - B)pq - \alpha y_0 + \beta x_0 & \frac{d\gamma}{dt} &= \alpha q - \beta p \end{aligned}$$

where (p, q, r) and (α, β, γ) are the components of angular velocity and the direction cosines (which define the orientation of the top) respectively. The variables (A, B, C) and (x_0, y_0, z_0) are the moments of inertia and the position coordinates of the centre-of-gravity respectively. These are the adjustable parameters of the system—for different values of which the system may or may not be integrable.

The solving, or integrating, of such a system of equations proceeds by identifying the integrals of the motion—a complete integration requires one to find as many of them as the order of the system (six in this case). These integrals are various analytical functions of the dependent variables that are constant in time. From fundamental physical considerations there are certain 'classical' integrals that can be identified immediately. These are the total energy, the angular momentum and, from simple geometric considerations, the sum $\alpha^2 + \beta^2 + \gamma^2$. As it turns out, with further standard simplifications, a complete solution of the problem requires the finding of only one more integral. The quest for this fourth integral became a popular problem in eighteenth and nineteenth century mathematics. However, it could only be found for three special cases: the trivial case of $A = B = C$, the case $x_0 = y_0 = z_0$ due to Euler and the case $A = B$, $x_0 = y_0 = 0$ due to Lagrange. A general solution to the problem seemed to be unobtainable.



Sofya Kovalevskaya from *A Russian Childhood* (ref. 6).

Kovalevskaya's approach⁸ to this problem was to use the mathematically powerful, but seemingly unphysical, techniques of complex variable theory. Apparently motivated by the work of Fuchs, she set out to determine the conditions for which the only movable singularities exhibited by the solutions to the equations of motion, in the complex time plane, would be ordinary poles. She found that this only occurred for four special combinations of the parameters (A, B, C) and (x_0, y_0, z_0) , namely the three known cases and any new one ($A = B = 2C$, $z_0 = 0$), for which she was able to find the fourth integral of motion and hence effect an integration of the equations of motion.

To understand the basis for this result, we must now examine in more detail the properties of functions of a complex variable and the theory of elliptic functions and their generalizations. Before we do this it is worth emphasizing that the original top problem is not a desiccated example of nineteenth century mathematics. More recently it has found useful applications in the theory of stationary flows of incompressible fluids⁴.

[Note that for linear ordinary differential equations the singularities are determined by the coefficients and are fixed. Thus the equation $(df/dz) + (1/z^2)f(z) = 0$, which has the solution $f(z) = Ce^{1/z}$, has a fixed (in this case, essential) singularity at $z = 0$. On the other hand, nonlinear ordinary differential equations can have movable singularities. Thus the equation $(df/dz) + f^2(z) = 0$, which has the solution $f(z) = (z + f^{-1}(0))^{-1}$, has a movable pole whose position is determined by the initial condition $f(0)$.]

Elliptic functions' theory

The elementary functions of classical analysis usually have a fairly simple structure when viewed in the complex plane. For example, functions such as $\sin(z)$ and $\cos(z)$ have no singularities in the (finite) complex z plane. Such functions are termed entire functions. An important theorem due to Liouville states that entire bounded functions can only take on one value, that is they are constant valued functions. From this we can immediately draw a simple but important conclusion of dynamical significance. The integrals of motion that are required to solve differential equations are constants of the motion. Therefore, they must be entire functions and, in mathematical terms, our ability to find integrals can be translated into our ability to construct entire functions.

Functions whose only singularities are ordinary poles are termed meromorphic. Elliptic functions are an example of such functions. They can arise as the solutions to certain types of nonlinear ordinary differential equations⁹. For example, the equation of motion for a particle (of unit mass) moving in the cubic potential $V(x) = -2x^3 + \frac{1}{2}g_2x$ (where the coefficients 2 and g_2 are chosen for notational convenience) is

$$\frac{d^2x}{dt^2} = 6x^2 - \frac{1}{2}g_2 \quad (1)$$

To integrate this equation we must find an integral of motion. We note that equation (1) can be reduced from a second-order to a first-order equation by expressing it in the form

$$\left(\frac{dx}{dt}\right)^2 = 4x^3 - g_2x - g_3 \quad (2)$$

as may be verified by differentiating through with respect to t and cancelling the common factor (dx/dt) . The new constant of integration g_3 is our desired (first) integral. We note that

$$-\frac{1}{2}g_3 = \frac{1}{2}\left(\frac{dx}{dt}\right)^2 - 2x^3 + \frac{1}{2}g_2x$$

is, in fact, just the mechanical energy (or hamiltonian) of the system. Equation (2) is now solved by the integration

$$t = \int^x \frac{dx'}{\sqrt{4x'^3 - g_2x' - g_3}} + C \quad (3)$$

where C is a constant of integration (the second integral). Proceeding from equations (1) to (3) is termed in the classical literature as 'integrating by quadratures' and is, loosely speaking, our notion of finding an 'exact' solution². Of course, in equation (3) we only have t as a function of x ; clearly we require the inverse function $x = x(t)$. This inversion is an elementary example of the Jacobi inverse problem¹¹.

Equation (3) is an example of an elliptic integral the inverse of which, in this case, is the famous Weierstrass elliptic function (usually denoted as $P(t)$). The function $t(x)$ is an infinitely multivalued function whereas $x = P(t)$ is a single valued function (compare with $y = \sin(x)$; y is a single valued function of x but $x = \sin^{-1}(y)$ is infinitely multivalued). Viewed in the complex x plane, the integrand in equation (3) has an interesting topological structure. The cubic expression can be factorized as $4x^3 - g_2x - g_3 = (x - e_1)(x - e_2)(x - e_3)$ where the roots e_1, e_2, e_3 are related to g_2 and g_3 . We can now see that the integrand is singular but, owing to the square root, these singularities are branch points. By pairing up two of these branch points and the third one with the point at infinity, the complex plane acquires two branch cuts. Although the complex plane (that is, the Riemann surface) has a complicated multisheeted structure, it can, with some careful 'cutting and pasting' along the branch cuts, be folded into a two-dimensional torus with a single hole—we term this a surface of genus one.

This takes us into the realms of algebraic geometry. Here one can associate with a given Riemann surface a certain irreducible polynomial which, in turn, defines an algebraic curve¹⁰. For

surfaces of genus one, the canonical form of the associated curve is $r^2 = 4s^3 - g_2s - g_3$. If we identify $s = P(t)$ and $r = dP(t)/dt$, we see that this is exactly the differential equation (2). From our point of view one of the most important results is that for curves of genus one (elliptic curves) the coordinates r and s are meromorphic functions of some parameter t . This geometric point of view becomes particularly valuable when we go on to consider much more general cases than equation (1).

Fortunately the meromorphic nature of the solutions to equation (1) may be determined in a rather direct way by examining their local properties. A meromorphic function can be expanded about a given singularity in the form of a Laurent series, that is

$$x(t) = \frac{1}{(t - t_0)^\alpha} \sum_{m=0}^{\infty} a_m(t - t_0)^m \quad (4)$$

where t_0 is the pole position and the 'leading order' α an integer. The validity of this expansion can be determined by direct substitution into the differential equation (1). By balancing most singular terms, it is easy to see that $\alpha = 2$ —thus the singularities are second order poles. An analysis of the expansion coefficients a_m , which can be determined recursively, shows that a_6 is arbitrary. This arbitrary coefficient plus the arbitrary pole position t_0 constitute the two pieces of arbitrary data required to specify the solution of a second-order differential equation (their actual values are determined by the initial conditions).

This test of local properties is rather powerful. For a more complicated system of equations, such as the Euler–Poisson equations, one makes the substitution (4) for each of the dependent variables ($p, q, r, \alpha, \beta, \gamma$). If they all have integer leading order and if the total number of arbitrary parameters (including the common pole position t_0) is enough (the order of the system), one has demonstrated that the movable singularities exhibited by the solution are ordinary poles. It was essentially this procedure that was used by Kovalevskaya. It can be used to good effect to study dynamical systems of current interest.

Note that while the Weierstrass elliptic functions have second-order poles ($\alpha = 2$) and the Jacobi elliptic functions (which arise when the right-hand side of equation (1) is replaced by a cubic polynomial) have first order poles ($\alpha = 1$), solutions to equation (1) with a right-hand side, including terms of higher than third order, have branch points. For example, if the right-hand side of equation (1) is a polynomial of order five, $f_5(x)$, then the leading order is easily seen to be $1/2$. Thus the movable singularities become movable branch points. The corresponding quadrature is now the hyperelliptic integral

$$t = \int^x \frac{dx'}{\sqrt{f_6(x)}} \quad (5)$$

The corresponding Riemann surface is now a surface of genus two (a torus with two holes) and the associated algebraic curve, a hyperelliptic curve, cannot be parameterized in terms of meromorphic functions. However, Jacobi observed that certain symmetrical combinations of hyperelliptic integrals do have meromorphic inverses.

In fact, the theory of hyperelliptic integrals and their inverses can be cast in a very general form and generalized to many dimensions. The integrals in equations (3) and (5) are just special cases of abelian integrals¹¹ (named after the Norwegian mathematician Niels Abel who died in poverty and relative obscurity aged 27). The associated Riemann surfaces are examples of what are termed abelian varieties. The right combinations of variables, which lead to abelian functions (Weierstrass and Jacobi elliptic functions are just special cases of these), are meromorphic functions. This is a result of particular significance in the study of dynamical systems. Unfortunately, the modern approach to abelian function tends to be very geometrical and hence not easily accessible to physical scientists. On the other hand, the old fashioned, analytical approach was (and is) often very dull. In a letter to Mittag-Leffler, Kovalevskaya wrote 'I

was simply disgusted, after having read, for example, the treatise on abelian functions of Briot. How is it possible to expose such a beautiful subject in so dry a manner to the students? (see ref. 7). Note that general theorems concerning Kovalevskaya's result (that is the connection between movable poles and integrability) have yet to be proved. Only recently have some results for a few special cases been found¹².

Painlevé and his contemporaries

An important way of classifying ordinary differential equations is according to the types of singularities that their solutions can exhibit. One of the most extensive investigations of this sort was carried out by the French mathematician Paul Painlevé at the turn of the century. He was an important political figure in Europe during the early part of the twentieth century—serving as French defence minister during World War I. He was very interested in aviation and as a friend of the Wright brothers became the first passenger (nonpaying) in aviation history. He moved in exclusive intellectual circles and his 'set' included the Clemenceaus and Gustav and Alma Mahler.

Following earlier work by L. Fuchs and others on the classification of first-order differential equations, he set about determining the categories of second-order equations whose only movable singularities are ordinary poles. Working with the general class of equations

$$\frac{d^2y}{dx^2} = F\left(\frac{dy}{dx}, y, x\right) \quad (6)$$

where F is analytical in x and rational in y and dy/dx , he found that there were 50 types which had the desired analytical property¹³. Forty-four of these equations could be solved in terms of known functions (including the elliptic functions). The remaining six equations, now usually referred to as the Painlevé transcendents, have transcendental meromorphic solutions. In fact, Painlevé himself only found the first two of the transcendents. These are

$$\frac{d^2y}{dx^2} = 6y^2 + x \quad (7)$$

$$\frac{d^2y}{dx^2} = 2y^3 + xy + \alpha \quad (8)$$

and the remaining four were found by various of his pupils. The sixth, due to Gambier, is the most general, embracing the other five as special cases of it. Note that whereas the differential equations with elliptic function solutions are algebraically integrable, that is their integrals are algebraic functions, the Painlevé transcendents are not. The significance of this, if any, is still not fully understood. It is fairly easy to show⁹ that, asymptotically, the solutions of the first and second transcendents behave like elliptic functions. However, unlike the latter, little is known about the actual distribution of poles in the complex plane of these equations.

There are some remarkable connections between nonlinear ordinary differential equations of the Painlevé type and certain properties of linear ordinary differential equations. R. Fuchs (son of L. Fuchs) studied the linear differential equation

$$\frac{d^2z}{dt^2} = z \left(\frac{\alpha}{t^2} + \frac{\beta}{(t-1)^2} + \frac{\gamma}{(t-x)^2} + \frac{\delta}{t(t-1)} + \frac{3}{4(t-y)^2} + \frac{a}{t(t-1)(t-x)} + \frac{b}{t(t-1)(t-y)} \right) \quad (9)$$

which has fixed singularities at $t=0, 1, \infty, x$ and y , where y is considered to be a function of the parameter x . He showed that as the singularity position x was varied, something called the monodromy group of the equation would be preserved, providing that certain conditions were met—in particular that $y(x)$ was a solution to the sixth Painlevé transcendent! This is, in fact, an example of a more general observation that many special

nonlinear ordinary and partial differential equations arise as the condition for the preservation of certain properties (for example, monodromy group or spectral properties) of linear equations under deformation.

As impressive as this body of work was (and is), at the time it appeared to have little application to the physical sciences. Indeed, there was apparently only one reference to the Painlevé transcendents in the contemporary non-mathematical literature¹⁴ (in connection with a certain problem in kinetics). Many scientists of the day were soon to be distracted by World War I and then by the advent of quantum mechanics and Hilbert's linear spaces. As a result, much of the work on nonlinear equations and deformation problems lay dormant. The situation has changed radically in the past 10 years or so.

Modern applications

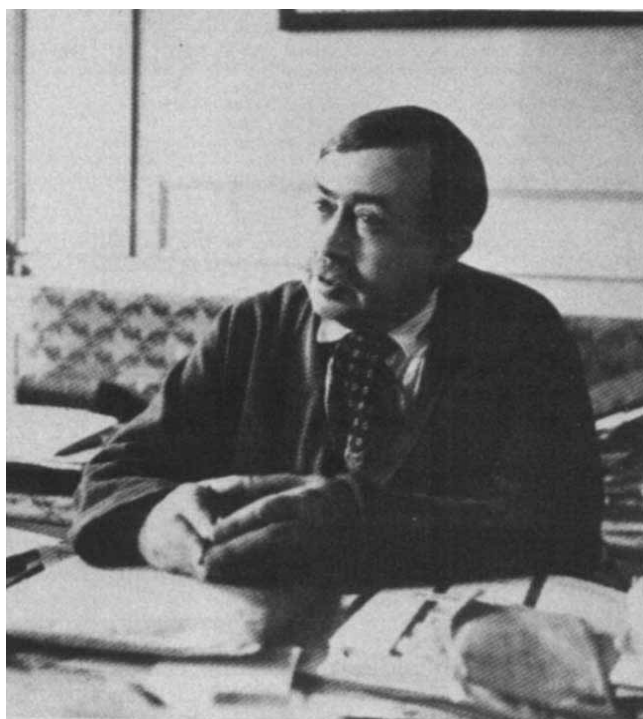
The Painlevé transcendents and differential equations with the 'Painlevé property', that is, equations whose solutions exhibit poles as their only type of movable singularities, are found in a variety of different fields including statistical mechanics, soliton theory and dynamical systems. One of the most striking (relatively) early occurrences was in statistical mechanics. Here it was found that in a certain limit the two-point correlation functions for the two-dimensional Ising model (spin lattice) had closed expressions in terms of the solutions of the third Painlevé transcendent¹⁵. Subsequently, similar results, in both classical and quantum field theories, with fascinating connections to deformation theory and some of the other Painlevé transcendents, have been found¹⁶.

In the study of dynamical systems, various (simple) systems of nonlinear ordinary differential equations have been studied extensively. For example, a simple model due to Henon and Heiles¹⁷, for the motion of a star in a gravitationally smoothed, cylindrically symmetrical galactic potential, takes the form of a pair of cubically coupled oscillators with equations of motion

$$\begin{aligned} \frac{d^2x}{dt^2} &= -Ax - 2Dxy \\ \frac{d^2y}{dt^2} &= -By - Dx^2 + Cy^2 \end{aligned} \quad (10)$$

These equations also serve as a simple model for studying the flow of energy between a pair of molecular bonds. This model was first studied in the case of the parameters $A = B = C = D = 1$ as a prototype of nonintegrable behaviour. Naturally, one would also like to know if there are any values of the parameters A, B, C, D for which the system (10) is integrable. By using Kovalevskaya's approach, that is looking for those parameter values for which the equations exhibit the Painlevé property, four integrable cases were identified¹⁸. Subsequently, all cases were shown to be integrable in terms of the Weierstrass elliptic functions¹⁹. In these cases, the poles in the complex t -plane are organized in a regular lattice typical of elliptic functions. In certain nonintegrable regimes, it was observed numerically and demonstrated analytically, that the singularities form up in remarkable self-similar (fractal) sets¹⁸. Another much studied system of differential equations is that due to Lorenz as a model for thermal convection²⁰. The nonintegrable behaviour of this system, with the appearance of a 'strange attractor', has excited much attention. From the analytical structure point of view, its integrable cases were identified by means of Painlevé property, and in the nonintegrable regions certain real time behaviours, such as turbulent 'bursts', can be correlated with changes in the distribution of singularities²¹.

Solitons are the remarkably stable, solitary wave-like solutions that are exhibited by certain nonlinear partial differential equations. The first recorded observation of a soliton is due to John Scott Russell in 1834. When riding by the Union Canal near Edinburgh he observed a large solitary water wave which persisted in its shape and speed for many miles. He wrote "This is a most beautiful and extraordinary phenomenon: the first day



Paul Painlevé from *Oeuvres de Paul Painlevé* (ref. 33).

I saw it was the happiest day of my life." At the time there was much debate as to whether such a wave could exist—according to linear theory it would not be stable. The question was resolved in 1895 by Kortweg and de Vries, who derived a nonlinear water wave equation of the form

$$u_t + 6uu_x + u_{xxx} = 0 \quad (11)$$

where $u = u(x, t)$ is the wave amplitude. This equation promptly disappeared and did not reappear until the 1960s in certain plasma physics contexts. Calculations then demonstrated that the travelling wave solutions to equation (11) were remarkably stable and it was at this point that the term 'soliton' was introduced. Most remarkable of all was the development by Kurikawa and co-workers of a new technique—the inverse scattering transform IST—to solve the KdV equation (11) (ref. 22). In fact, this technique is an example of a deformation equation. For the following linear eigenvalue problem (the Schrödinger equation)

$$-\frac{1}{2} \frac{\partial^2 \psi}{\partial x^2} + (u(x, \alpha) - \lambda) \psi = 0 \quad (12)$$

under what deformations of the potential $u(x; \alpha)$, where α is some parameter, will the spectrum of eigenvalues, λ , remain invariant? What one finds is that $u(x; \alpha)$ must vary according to the differential equation $u_\alpha + 6uu_x + u_{xxx} = 0$ which is exactly the KdV equation (11).

Following the studies of the KdV equation, other nonlinear partial differential equations, with applications to nonlinear optics, theory of water waves, plasma physics and so on have been found that can be solved by IST and exhibit soliton type solutions^{23,24}. Such equations are termed 'completely integrable' and, corresponding to their infinite number of degrees of freedom, have an infinite number of integrals of motion. There are many interesting connections between integrable partial differential equations and (integrable) ordinary differential equations of the Painlevé type. A typical example of such a connection is provided by the modified KdV equation

$$u_t - 6u^2u_x + u_{xxx} = 0 \quad (13)$$

which can be solved by IST. It can be reduced to an ordinary differential equation by the similarity transform $u(x, t) =$

$(3t)^{-2/3} f(z(3t)^{-1/3})$. The function $f(z)$ is found to satisfy

$$\frac{d^2 f}{dz^2} = 2f^3 + 2f + \alpha \quad (14)$$

which is just the second Painlevé transcendent. Another interesting property of some completely integrable systems, which emphasizes their analytical structure, is the representation of their solution in terms of poles^{25,26}. For example, the solution to the KdV equation (12) can be represented as

$$u(x, t) = -2 \sum_{i=1}^n \frac{1}{(x - x_i(t))^2} \quad (15)$$

where the poles $x_i(t)$ are found to evolve, with certain constraints, according to the integrable system of ordinary differential equations

$$\frac{dx_i}{dt} = -144 \sum_{\substack{j=1 \\ (j \neq i)}}^n \frac{1}{(x_i - x_j)^5} \quad (16)$$

Clearly, given a set of nonlinear partial differential equations, it would be most desirable to have a direct test of integrability—and hence the possibility of solitons—rather than having to solve the equations completely. One valuable suggestion has been to look for connections with ordinary differential equations with the Painlevé property (by means of similarity transforms and other types of reduction)^{27–29}. Of course, in the complex plane, functions of many complex variables (that is solutions to partial differential equations) have a more complicated structure than functions of one complex variable. In particular, the singularities are no longer isolated points but form surfaces ('singular manifolds') on which the solutions blow up. Recently, it has been suggested that a multidimensional generalization to the Painlevé property could be provided by looking for solutions that have local single-valued expansions about their movable singular manifolds (a generalized notion of meromorphicity)³⁰. An attractive feature of this approach is that it can be applied directly to a given partial differential equation without the need to reduce it to an ordinary differential equation. This procedure seems to work and, furthermore, has nice connections with existing procedures.

The singularity structure of nonintegrable systems can also be important. For example, there is some evidence that the equations for three-dimensional, inviscid, incompressible fluid flow (the Euler equations) may exhibit real time singularities³¹. In the presence of viscosity (the Navier–Stokes equations), these singularities will be lifted off the real axis but their presence could be responsible for the onset of turbulence and intermittency³².

Hopefully, this survey has demonstrated that a very wide range of equations can be treated in a fairly unified way by examining their analytical structure. But much more work needs to be done.

I thank David and Gregory Chudnovsky for many valuable historical insights. This work is supported in part by the Office of Basic Energy Sciences of the US Department of Energy.

1. Percival, I. C. & Richards D. *Introduction to Dynamics* (Cambridge University Press, 1982).
2. Whittaker, E. T. *A Treatise on the Analytical Dynamics of Particles and Rigid Bodies* 4th edn (Cambridge University Press, 1959).
3. Born, M. *The Mechanics of the Atom* (Ungar, New York, 1960).
4. Arnold, V. I. *Mathematical Methods of Classical Mechanics* (Springer, New York, 1978).
5. Lichtenberg, A. J. & Lieberman, M. A. *Regular and Stochastic Motion* (Springer, New York, 1982).
6. Kovalevskaya, S. *A Russian Childhood* (ed. Stillman, B.) (Springer, New York, 1978).
7. Golubev, V. V. *Lectures on Integration of the Equation of Motion of a Rigid Body About a Fixed Point* (State Publishing House, Moscow, 1953).
8. Kovalevskaya, S. *Acta math.* **12**, 177–232 (1889); **14**, 81–83 (1889).
9. Davis, H. T. *Introduction to Nonlinear Differential and Integral Equations* (Dover, New York, 1960).
10. Hille, E. *Analytic Function Theory* (Chelsea, New York, 1982).
11. *Encyclopedic Dictionary of Mathematics*, (MIT Press, 1980).
12. Adler, M. & van Moerbeke, P. *Invent. Math.* **67**, 297–331 (1982).
13. Ince, E. L. *Ordinary Differential Equations* (Dover, New York, 1956).
14. Jüttner, F. *Z. math. Phys.* **58**, 385–409 (1910).
15. Wu, T. T., McCoy, B. M., Tracy, C. A. & Barouch, E. *Phys. Rev. B* **13**, 316–374 (1976).
16. Jimbo, M., Miwa, T., Mori, Y. & Sato, M. *Physica D* **1**, 80–158 (1980).

17. Henon, M. & Heiles, C. *Astr. J.* **69**, 73–79 (1964).
18. Chang, Y. F., Tabor, M. & Weiss, J. *J. math. Phys.* **24**, 531–538 (1982).
19. Weiss, J. *Phys. Lett.* **102A**, 329–331 (1984).
20. Lorenz, E. N. *J. atmos. Sci.* **20**, 130–141 (1963).
21. Tabor, M. & Weiss, J. *Phys. Rev. A24*, 2157–2167 (1981).
22. Miura, R. M. *SIAM Rev.* **18**, 412–459 (1976).
23. Ablowitz, M. J. & Segur, H. *Solitons and the Inverse Scattering Transform* (SIAM, Philadelphia, 1981).
24. Dodd, R. K., Eilbeck, J. C., Gibbon, J. D. & Morris, H. C. *Solitons and Nonlinear Wave Equations* (Academic, London, 1982).
25. Airault, H., McKean, H. P. & Moser, J. *Commun. Pure appl. Math.* **30**, 95–198 (1977).
26. Chudnovsky, D. V. & Chudnovsky, G. V. *Nuovo Cimento* **40B**, 339–353 (1977).
27. Ablowitz, M. J., Ramani, A. & Segur, H. *J. math. Phys.* **21**, 715–721 (1980).
28. Nakach, R. *Plasma Physics, Proc 36th Nobel Symp.* (ed. Wilhelmsson) (Plenum, New York, 1977).
29. McLeod, J. B. & Olver, P. J. *SIAM J. math. Anal.* **14**, 488–506 (1983).
30. Weiss, J., Tabor, M. & Carnevale, G. *J. math. Phys.* **24**, 522–526 (1983).
31. Morf, R. H., Orszag, S. A. & Frisch, U. *Phys. Rev. Lett.* **44**, 572–575 (1980).
32. Frisch, U. & Morf, R. H. *Phys. Rev. A23*, 2673 (1981).
33. *Oeuvres de Paul Painlevé* Vol. 1 (Centre National de la Recherche Scientifique, Paris, 1973).

ARTICLES

Turbulence in an oceanic convective mixed layer

Thomas J. Shay & Michael C. Gregg

Applied Physics Laboratory and School of Oceanography, University of Washington, Seattle, Washington 98105, USA

The first direct measurements of turbulence in an oceanic convective mixed layer were obtained during a cold-air outbreak. The uniformity of the turbulent dissipation rate and its relation to the surface buoyancy flux are similar to observations of convection in the atmosphere.

CONVECTION is one of the most important, yet least understood, mechanisms by which heat is transferred between the atmosphere and ocean, and between the upper ocean and the deep ocean. At high latitudes, convection reaches great depths and is responsible for the formation of much of the intermediate and bottom water¹. At mid-latitudes, convection modulates the depth and homogeneity of the upper ocean on daily and seasonal cycles^{2,3}. In spite of the importance of convective mixing, previous turbulence measurements had been made only in shallow oceanic mixed layers driven by the wind stress, not by the surface buoyancy flux⁴. Further progress in modelling upper ocean dynamics requires a better understanding of the effects of convection, particularly in dissipating turbulent kinetic energy within mixed layers⁵.

An experiment to measure convective turbulence was performed in January 1983. To obtain maximum convective forcing, the measurements were made within warm-core Gulf Stream ring 83-I (41° N 66° W) during a cold-air outbreak. Warm-core rings are formed when northward meanders of the Gulf Stream pinch off, enclosing warm water from the Sargasso Sea. During January, sea-surface temperatures within ring 83-I were 5–8 °C warmer than the surrounding slope water. In winter, outbreaks of Arctic air over eastern North America often follow the passage of low-pressure centres. As this cold air flows over the ocean, large air-sea temperature differences and surface heat fluxes are produced in warm-core rings.

This analysis examines the characteristics of ϵ , the rate of dissipation of turbulent kinetic energy, within the mixed layer. The observations are interpreted within the context of the atmospheric forcing and the bulk response of the ocean to that forcing. Finally, the results are compared with observations made in atmospheric convective mixed layers.

Atmospheric forcing and bulk response

Direct atmospheric forcing of the upper ocean is produced by the work of the wind stress, E_w , and by the surface buoyancy flux, J_b^0 . These parameters were computed using routine observations in the ship's weather log and bulk aerodynamic formulae^{6–8} and are shown in Fig. 1. The wind forcing was dominated by two storms. A cold-air outbreak followed the second storm, producing large heat fluxes and rapid deepening of the mixed layer.

The major forcing by the wind stress occurred during short bursts of high winds during the storms. The maximum value of E_w , 19 W m⁻², occurred on 17 January and was produced by winds with speeds greater than 20 m s⁻¹. E_w then dropped to

<2 W m⁻² which, nevertheless, represents significant wind forcing and corresponds to the largest magnitudes reported from the Joint Air-Sea Interaction Experiment (JASIN)⁹.

Because the sea-surface temperature within the ring varied by <2 °C, fluctuations in the heat and buoyancy fluxes were due to changes in air temperature and wind speed. A mild decrease in air temperature followed the first storm, producing air-sea contrasts of up to 5 °C. A much larger air temperature decrease began on 17 January, during the second storm, and continued to 21 January. Air-sea temperature contrasts of 15 °C produced upward surface heat fluxes, J_q^0 , of 600 W m⁻² during the 'lull' in the winds. The heat flux was due equally to sensible and latent components, and the corresponding surface buoyancy flux, J_b^0 , was 2.8×10^{-7} W kg⁻¹. A short burst of high winds on 20 January increased J_q^0 to 900 W m⁻² and J_b^0 to 5×10^{-7} W kg⁻¹.

The Monin-Obukhov length, $L = -u_*^3 / \kappa J_b^0$ (where u_* is the friction velocity and κ is von Karman's constant), gives the relative strength of the two types of forcing. L is negative in convective conditions, and $|L|$ is the depth scale at which wind stress and buoyancy flux are equally effective in producing turbulence within the mixed layer. Thus, convection should be the dominant mechanism for $|z/L| > 1$, if $|L|$ is less than the mixed layer depth, D . The first storm increased D from 15 m to between 35 and 50 m (Fig. 1d). Subsequently, the layer eroded until the arrival of the second storm. When the ship returned to the ring centre, late on 18 January, D was ~75 m and increasing rapidly, reaching 160–170 m by 20 January. During this time, $5 < D/L < 10$, and convective forcing was dominant throughout most of the mixed layer. The frequent occurrence of snow and sea smoke and the agitated condition of the sea surface obscured any surface patterns that may have been produced by subsurface convection.

Stratification and shear

Mixing results from an interaction between forcing and the static and dynamic states of the water column. In particular, the stratification (given by the stability frequency, N), the temperature-salinity (T_s) relationship, and the shear affect the response to forcing. These parameters are shown in Fig. 2 for one of the first and one of the last bursts of profiles in the ring.

Before the first storm, a salt-stabilized temperature inversion extended from the base of the shallow mixed layer to a temperature maximum near 2 MPa. The ring had apparently been flooded by cooler, less saline slope water¹⁰. Owing to the nearly compensating effects of temperature and salinity on density, the mean stratification was weak through most of the temperature

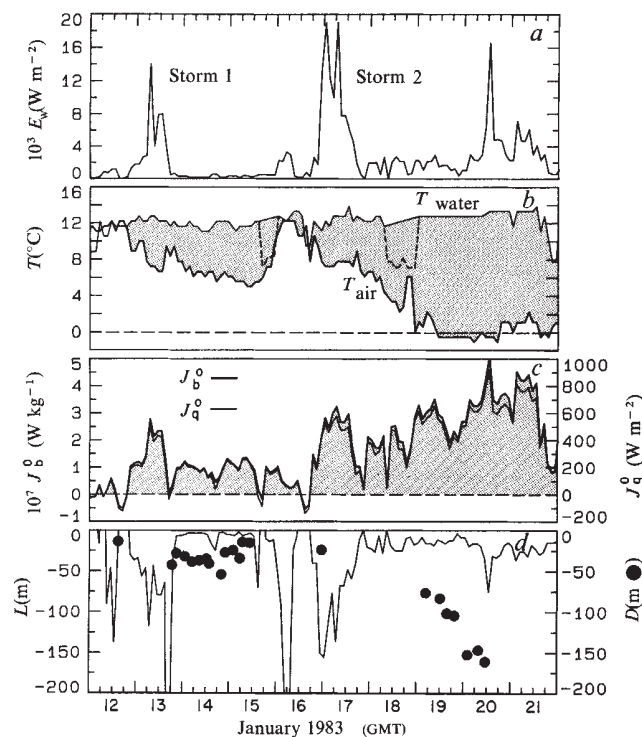


Fig. 1 Summary of atmospheric forcing, showing the passage of two storms, the cold-air outbreak following the second storm, and the deepening of the mixed layer. The meteorological variables were computed from observations entered every 2 h in the ship's weather log. $E_w = \rho u_*^3$ is the rate of work by the wind on the sea surface (ρ is the density of water and u_* is the oceanic friction velocity); J_b^0 and J_q^0 are the surface fluxes of buoyancy and heat, respectively; L is the Monin-Obukhov length; and D is the mixed layer depth. The sea-surface temperature depressions (dashed line) on 15 and 17 January were caused by the temporary movement of the ship to the edge of the ring. J_b^0 , J_q^0 and L were calculated using interpolated sea-surface temperatures on 15 and 17 January (solid line) so that they would be representative of conditions within the ring.

inversion. Strong density gradients existed only between 0.1 and 0.3 MPa and below 1.4 MPa (Fig. 2a). Hence, once the mixed layer penetrated the upper stratified zone, the work required for further deepening decreased until the stratified region near 1.4 MPa was reached.

The initial Ts relation was unstable to the diffusive regime of double diffusion, which is most likely to occur when $R_p \equiv \beta s_z / \alpha T_z < 2$ (α and β are the coefficients of thermal expansion and haline contraction, respectively). From the surface to the Ts maximum near 2 MPa, $R_p \approx 1.5$, and numerous instances of double diffusion have been found in the profiles. Some of these have been reported previously¹¹; in the present context, double diffusion is considered only as part of the background mixing beneath the mixed layer. Because the maximum values of ϵ attributable to double diffusion are only 20% of the mean values in the mixed layer and occur only in regions several metres thick, their effect on the dynamics of the mixed layer is negligible.

Shear was monitored with 25 velocity profiles taken with Expendable Current Profilers (XCP)¹² which have a vertical resolution of about 10 m. Before the second storm, the strongest vertical shear was generally found below the base of the mixed layer (a typical example is shown in Fig. 2a), but the presence of even small shear within the upper 1.5 MPa was significant because the stratification was so weak. Despite the low values of N between 0.3 and 1.3 MPa, the Richardson number, $Ri = [N/(\partial U/\partial z + \partial V/\partial z)]^2$ where U and V are velocity components, determined from individual XCP profiles, was < 0.25 only in the high shear region above 0.6 MPa; the stratification below 0.6 MPa was sufficient to prevent shear instability. After the

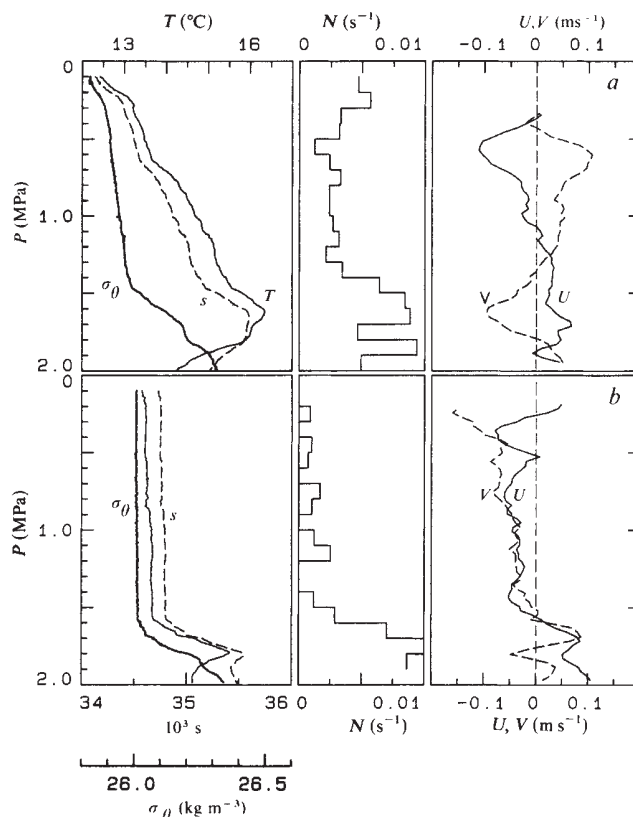


Fig. 2 The deepening of the mixed layer is shown by profiles taken before and after the cold air outbreak. (A sea pressure of 1.0 MPa corresponds to a depth of 100 m.) a, Average vertical profiles of temperature (T), salinity (s), density (σ_θ) and buoyancy frequency (N) from AMP burst 14 (0900–1000 GMT 15 January) and U and V components of velocity from XCP 1156 (1220 GMT 15 January). b, Profiles from AMP burst 34 (0700–0830 GMT 20 January) and XCP 1171 (0540 GMT 20 January). U and V are centred on their mean values.

cold-air outbreak, the upper 1.5 MPa of the water column was well mixed and Ri was < 0.25 throughout (Fig. 2b). Below 1.5 MPa, the stratification remained large enough to compensate for the shear and Ri was about 0.8.

Dissipation in the deepening mixed layer

During the cold-air outbreak, vigorous mixing and high dissipation rates were observed throughout the mixed layer. Direct measurements of dissipation-scale velocity fluctuations were made with two airfoil lift probes¹³ mounted on the Advanced Microstructure Profiler (AMP)¹⁴. The dissipation rates, ϵ , were calculated directly by integrating velocity gradient spectra and assuming spectral isotropy.

Although much variability occurred in the 100 AMP profiles taken during the cold-air outbreak, three distinct regimes appeared in most of the profiles. The surface zone, 10–25 m thick, contained intermittent density inversions and ϵ values of 10^{-6} W kg^{-1} and higher. A well-mixed central zone, 50–160 m thick, extended to the density step at the base of the mixed layer. Within the central zone, ϵ was uniform with depth and had a mean value near 10^{-7} W kg^{-1} . Below the base of the mixed layer, a transition zone, up to 15 m thick, contained intermittent density inversions and ϵ decreased to background levels of 10^{-9} W kg^{-1} .

An example from an early stage of deepening is shown in Fig. 3a. In this profile, the surface zone extended to ~ 0.20 MPa and had $\epsilon > 10^{-6}$ W kg^{-1} , but contained no large density inversions. The rest of the mixed layer had nearly uniform ϵ levels

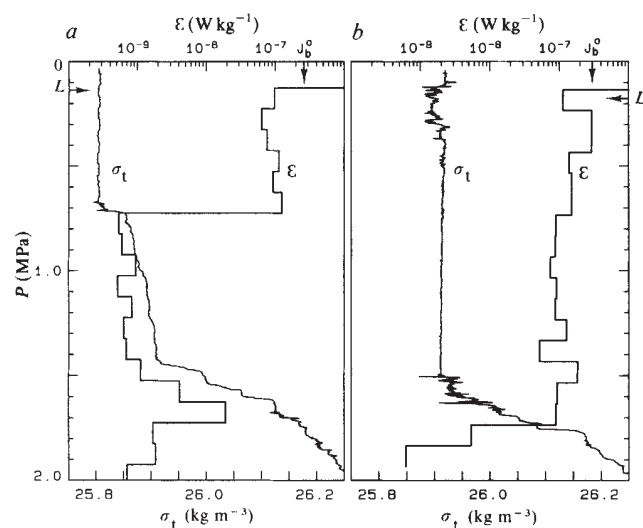


Fig. 3 Uniform dissipation rates through the mixed layer at two stages of the cold-air outbreak. *a*, AMP 1281 (0200 GMT 19 January). *b*, AMP 1360 (0500 GMT 20 January). The surface buoyancy flux (J_b^0) and Monin-Obukhov length (L) at the times of the profiles are indicated by the arrows on the ϵ and pressure axes. The profiles began at pressures of ~ 0.03 MPa.

of $1 \times 10^{-7} \text{ W kg}^{-1}$ which then decreased by a factor of 100 across the sharp density step at the base of the layer. The dissipation rates beneath the mixed layer are characteristic of those in the thermocline¹⁵, except for the increase between 1.5 and 1.8 MPa. This occurred in a sequence of steps of increasing temperature and salinity (not shown) and has been attributed to mixing in the diffusive regime of double diffusion¹¹.

The profile in Fig. 3*b* was taken early on 20 January, shortly after the mixed layer had penetrated into the more stratified region below 1.4 MPa. Density inversions as thick as 15 m were found above 0.4 MPa, and dissipation rates in the surface zone remained extremely high. From 0.4 MPa to the base of the layer at 1.5 MPa, ϵ was uniform at $2 \times 10^{-7} \text{ W kg}^{-1}$. In this record, ϵ did not diminish to background levels until 15 m below the base of the mixed layer. Overturns a few metres thick showed that water from the stratified section was being entrained.

The unique feature of these profiles is the relative uniformity of ϵ throughout mixed layers as deep as 180 m. This resembles those atmospheric convective mixed layers which have been studied extensively using sensors on towers, tethered balloons, and aircraft^{16,17}. Observations made over heated land show that, at heights where the absolute value of $z/L > 1$, the turbulent kinetic energy balance is between buoyant production and viscous dissipation¹⁸:

$$\epsilon = J_b \quad \text{W kg}^{-1} \quad (1)$$

To obtain better statistical significance in comparing our ϵ profiles with those from the atmosphere, ensemble averages were formed of seven bursts of drops taken during the cold air outbreak. Each burst contained 4–12 profiles and was taken during an interval of between 1 and 3 h. The seven bursts spanned a period of 27 h when the mixed layer depth increased from 70 to 170 m. Only data from depths greater than L were included in the averages, and no attempt was made to reject data from periods when the velocity shear was large. The results are shown (Fig. 4*a*) as dissipation profiles nondimensionalized with D and J_b^0 . For $0 < z/D < 0.35$, ϵ/J_b^0 often remained > 1 , reflecting contamination by the high turbulence levels in the wave zone. For $0.35 < z/D < 1$, $\epsilon/J_b^0 \approx 0.3$. At $z/D \approx 1.1$, ϵ/J_b^0 decreased by a factor of 100.

Dimensionless dissipation profiles from the atmospheric mixed layer are shown in Fig. 4*b*. The data are from the Minnesota experiment, performed over a flat, uniform wheat field¹⁶, and the Aschurich experiment, performed over less homogeneous

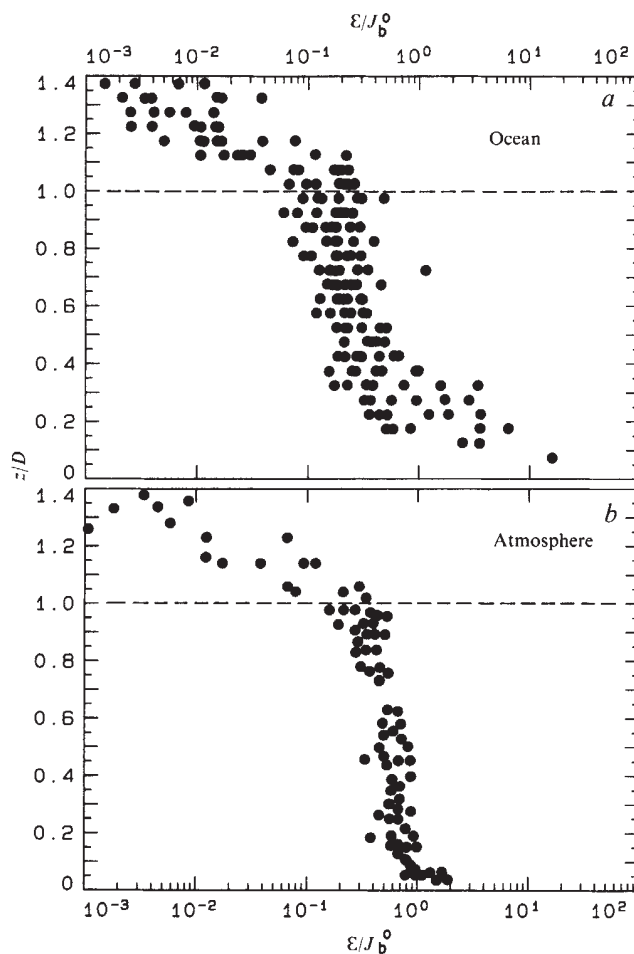


Fig. 4 Dimensionless dissipation profiles from the warm-core ring (*a*) compared with profiles from the atmospheric mixed layer (*b*). In this comparison, the sea surface is at the bottom of each plot. (Atmospheric data are replotted from ref. 17.) The AMP data from individual profiles were nondimensionalized, averaged vertically into bins $0.05D$ wide, and then averaged across 4–12 drops in each of seven bursts. The surface zone in the ocean, $0 < z/D < 0.35$, is relatively much thicker than in the atmosphere, $0 < z/D < 0.05$. In both data sets, ϵ decreases by several decades near $z/D = 1$.

fields¹⁷. Mixed layer heights were 500–2,500 m, and L varied from 5 to 50 m. Each point represents an average over 15 to 75 min, and the data were carefully edited to reject runs from periods with significant wind shear. (We have replotted the data from Fig. 9 of ref. 17.) The mixed layer dissipation remains relatively uniform at $\epsilon/J_b^0 \approx 0.6$ until a decrease of two to three orders of magnitude at $z/D \approx 1.0$.

The two profiles in Fig. 4 are similar: ϵ/J_b^0 remains uniform through most of the mixed layer and then decays rapidly beginning near $z/D = 1$. In the atmosphere, ϵ/J_b^0 in the mixed layer is larger by about a factor of 2. The significance of this difference is difficult to assess; the error in our fluxes, which were estimated using standard shipboard meteorological observations, is uncertain. Despite the uncertainty of the numerical comparison, the similarity of the profiles in Fig. 4 supports the interpretation of these data as evidence of convective turbulence in the ocean.

Conclusion

The data summarized in Fig. 4 provide the first direct evidence of convective turbulence in an oceanic mixed layer. A large heat flux, produced by a large air-sea temperature contrast and moderate winds, resulted in Monin-Obukhov lengths that were small compared with the deep mixed layer. Consequently, a zone 50–120 m thick existed in which the buoyancy flux was the dominant source of turbulence.

In the only other experimental study of mixed layer turbulence⁴, the mixing was predominantly wind-driven. The mixed layer reached a maximum depth of 25 m and the mean wind speed was 10 m s^{-1} . A surface heat flux of 350 W m^{-2} would have been required to produce $D/L = 1$. Because such a large heat flux is unlikely at that site, $D/L < 1$, and the turbulence throughout the mixed layer was wind-driven.

Convective mixed layer forcing similar to that reported here may be expected where warm surface waters are exposed to cold-air outbreaks. In winter, these conditions are found in

much of the northwestern Atlantic, the northwestern Pacific and the northern Gulf of Mexico.

This work was supported by the Office of Naval Research and the Naval Ocean Research and Development Activity. We thank D. Hirt, E. Kunze, N. Larson, P. McKeown, M. Nicholas and W. Nodland for working in difficult conditions to ensure the success of this experiment, and T. Joyce and M. Stalcup for mapping the large-scale features of the ring. School of Oceanography, University of Washington, contribution no. 1379.

Received 6 March, accepted 11 May 1984.

1. Warren, B. A. in *Evolution of Physical Oceanography* (eds Warren, B. A. & Wunsch, C.) 6-41 (MIT Press, Cambridge, 1981).
2. Shay, T. J. & Gregg, M. C. *EOS* **63**, 1002 (1982).
3. Gill, A. E. & Turner, J. S. *Deep-sea Res.* **23**, 391-401 (1976).
4. Oakey, N. S. & Elliott, J. A. *J. phys. Oceanogr.* **12**, 171-185 (1982).
5. Niiler, P. P. & Kraus, E. B. in *Modeling and Prediction of the Upper Layers of the Ocean* (ed. Kraus, E. B.) 143-172 (Pergamon, Oxford, 1977).
6. Large, W. G. & Pond, S. *J. phys. Oceanogr.* **11**, 324-336 (1981).
7. Large, W. G. & Pond, S. *J. phys. Oceanogr.* **12**, 446-462 (1982).
8. Smith, S. D. & Dobson, F. W. *Atmosphere-Ocean* (submitted).
9. Guymer, T. H. *et al. Phil. Trans. R. Soc. A* **308**, 253-273 (1983).
10. Stalcup, M. C., Joyce, T. M. & Barbour, R. L. *USNS Bartlett Cruise 40-B Data Rep.* (Woods Hole Oceanogr. Inst. Tech. Rep. WHOI-83-16 1983).
11. Larson, N. G. & Gregg, M. C. *Nature* **306**, 26-32 (1983).
12. Drever, R. G. & Sanford, T. B. *Near Surface Ocean Experimental Technology Workshop Proc.*, 163-173 (Naval Ocean Research and Development Activity, NSTL Station, Mississippi, 1980).
13. Osborn, T. R. & Crawford, W. R. in *Air-Sea Interaction* (eds Dobson, F. Hasse, L. & Davis, R.) 369-386 (Plenum, New York, 1980).
14. Gregg, M. C., Nodland, W. E., Aagaard, E. E. & Hirt, D. H. in *Oceans '82 Conf. Rec.* 260-265 (Marine Technology Society, Washington DC, 1982).
15. Lueck, R. G., Crawford, W. R. & Osborn, T. R. *J. phys. Oceanogr.* **13**, 1809-1818 (1983).
16. Kaimal, J. C. *et al. J. atmos. Sci.* **33**, 2152-2169 (1976).
17. Caughey, S. J. & Palmer, S. G. *Q. Jl R. met. Soc.* **105**, 811-827 (1979).
18. Caughey, S. J. & Wyngaard, J. C. *Q. Jl R. met. Soc.* **105**, 231-239 (1979).

Three-dimensional reconstruction of rigor insect flight muscle from tilted thin sections

Kenneth A. Taylor, Mary C. Reedy, Leonidas Córdova & Michael K. Reedy

Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710, USA

Rigor cross-bridges show two conformations paired within each 38.7-nm axial repeat. The two forms may express two stages of the cross-bridge cycle during contraction. Differing numbers of myosin heads per cross-bridge and associated helical changes in the thin filament distinguish the two forms and suggest that both myosin heads usually bind to a single thin filament.

THE primary molecular event in muscle contraction is generally believed to be the ATP-driven ratchet-like action of myosin cross-bridges upon adjacent actin filaments, causing filament sliding and sarcomere shortening. The structural response of cross-bridges to the different physiological states has been most clearly seen in flight muscle from the large waterbug *Lethocerus* sp., both by electron microscopy¹⁻³ and in X-ray diffraction patterns⁴⁻⁶.

We are concerned here with rigor, in which stable cross-bridge attachment to actin is maximized following ATP withdrawal. Rigor structure illuminates three questions of importance: (1) the shape(s) of the cross-bridges uniformly trapped at the end of the rigor power-stroke that produces tension during (and sustains it after) rigor induction, (2) the range of cross-bridge structures as constrained by filament geometry and myosin flexibility and (3) the helical and periodic structure of thick and thin filaments as expressed by the distribution of cross-bridge attachments. We expect that general features of cross-bridge structure can be distinguished from the particulars of grouping and lattice position encountered in insect flight muscle.

The computer three-dimensional reconstruction of the filament-cross-bridge lattice reported here was stimulated by the promise and limitations in previous efforts^{2,7}. Recently, cross-bridge structure has been deduced with the aid of optically filtered images from 15-nm cross-sections and multiple tilt views of single filament layers in longitudinal sections⁸. That study reveals two characteristically different shapes and attachment angles for rigor cross-bridges. Moreover, the actin azimuth at the cross-bridges can be inferred and is compatible with Taylor-Amos binding geometry⁹. Also, the cross-bridges appear large enough to accommodate two myosin heads.

However, because the structures can only be viewed in projection, it is possible that the two cross-bridge structures and angles

are more apparent than real. Moreover, optical filtering does not provide a quantitative basis for assigning molecular boundaries when determining cross-bridge size. To resolve these questions we have carried out a computer three-dimensional reconstruction as the next phase in structural analysis of *Lethocerus* flight muscle. This approach should also enable actin-myosin binding geometry to be determined decisively with thin section methodology *in situ*, complementary to data from negative-stained preparations of actin filaments decorated with myosin subfragment 1 (S-1).

The MYAC layer reconstruction

The reconstruction reported here is of the rigor MYAC layer, a single filament layer consisting of alternating thick and thin filaments found in thin longitudinal sections (Fig. 1). Glutaraldehyde-tannic acid fixation was used, as this has been found to give better preservation of filament spacing, lattice order, cross-bridge detail and actin substructure⁸. The three-dimensional reconstruction was done by combining image data from two tilt series using methods developed for reconstructing images of thin two-dimensional crystalline arrays¹⁰. The data combine well in space group p12 (Table 1, Fig. 2), indicating that at the resolution limit imposed by the sampled diffraction, approximate 2-fold rotation symmetry is present⁵. The resolution of the diffraction data is anisotropic and extends to 5.8 nm equatorially and 12.9 nm axially in the untilted view. The highest resolution data in the three-dimensional reconstruction extend to 4.6 nm.

The density map calculated for the full 116-nm axial repeat period has three groupings of paired cross-bridges, usually referred to as double chevrons (Fig. 3). Positive staining dominates all features, with no sign of the negative staining sometimes

Table 1 Three-dimensional tilt data set

Image	Tilt axis	Tilt angle	Phase residual
1,021	5.4	0.0	11.8
1,022	5.4	57.1	20.7
1,023	5.4	-59.4	22.9
1,024	5.4	-54.4	15.0
1,025	5.4	46.5	12.3
1,026	5.4	36.5	11.2
1,027	5.4	-44.4	10.7
1,028	5.4	-34.4	9.8
1,029	5.4	26.2	13.4
1,030	5.4	17.2	10.8
1,031	5.4	-24.8	10.9
1,032	5.4	-15.1	10.0
1,033	5.4	4.3	9.7
1,035	92.3	-55.8	34.8
1,036	92.3	52.1	34.3
1,037	92.3	42.4	15.2
1,038	92.3	-39.1	18.4
1,039	92.3	-29.3	15.2
1,040	92.3	32.6	12.6
1,041	92.3	23.3	13.4
1,042	92.3	-19.4	14.6
1,043	92.3	-11.9	9.4
1,044	92.3	12.4	9.9
1,045	92.3	0.0	7.9

Results from the origin refinement step merging 24 micrographs from two tilt series are shown. The first tilt series was collected starting with the highest tilt angles first, because the data from these are most affected by any section thinning caused by prolonged electron irradiation⁴⁰. There is, however, no evidence that such thinning actually occurred. Tilt axis represents the angle between the *a* axis (or equator) and the tilt axis of the electron microscope specimen stage. Micrographs were recorded at $\times 17,000$ magnification on a Philips EM400 equipped with high-tilt goniometer stage and rotation holder. The particular area of thin section used for the three-dimensional reconstruction was chosen where the two planes of sectioning appeared symmetrically placed and parallel about the filament axis and where the filaments within the section were parallel to one another. Two-dimensional scans were made using effectively a 1.7-nm raster. Fourier transforms of 256×256 point arrays were calculated using software written by Dr Linda Amos and run on an IBM 3081.

seen after tannic acid fixation¹¹. The three-dimensional arrangement of cross-bridges generates the characteristic left-handed helical pattern about the thick filament axis⁷. The thick filament backbone is an extremely smooth feature of average diameter 20 ± 2 nm, a value consistent with four myosin molecules per 14.5 nm level and 36–40 myosin tails packed within any cross-section^{12,13}. The thick filament backbone shows just a hint of the hollow centre often seen in electron micrographs³. An interdoublet gap 12.5–14.5 nm long separates double chevrons. An intradoublet gap 4.5 nm long at the thin filament suggests that one unoccupied actin monomer on average separates the lead and rear chevrons.

An exact alignment between the 14.5-nm myosin filament and the 38.7-nm thin filament axial periods can occur only every 116 nm. Therefore there should be structural differences among double chevrons within a MYAC layer; this is often seen in electron micrographs as a density variation in every third transverse row of double chevrons (see below). Diffraction data for the MYAC layer discussed here, however, did not show any orders requiring indexing to a 116-nm period other than a weak 14.5-nm meridional, shown here in the optical diffraction pattern (Fig. 1b). The density maps show no distinct feature associated with a 14.5-nm meridional repeat. It is possible, however, to determine where the 14.5-nm reflection is contributing density to the map (Fig. 3), an exercise that reveals no obvious correlation between it and either the thin filament marking or the cross-bridge origins on the thick filament.

Two structurally different cross-bridges

The most obvious feature of the three-dimensional density map is the different tilt of the lead and rear bridges within each

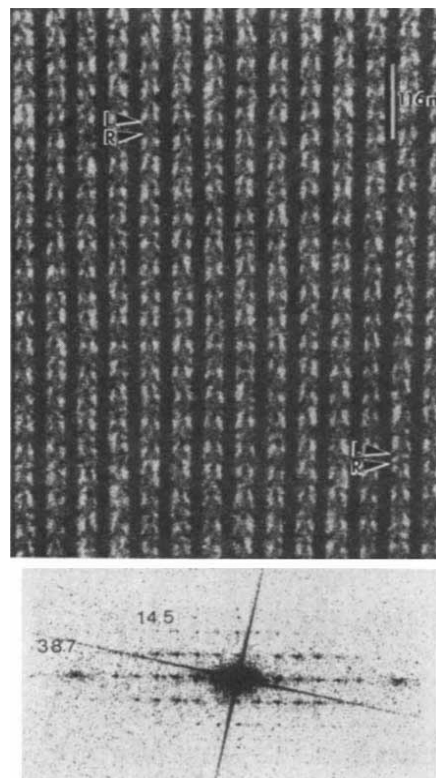


Fig. 1 *a*, Electron micrograph of the untitled view of the rigor MYAC layer used for this three-dimensional reconstruction. X-ray monitored fixation-embedding of a bundle of glycerinated *Lethocerus* flight muscle involved sequential tannic-acid-glutaraldehyde (0.2% and 2%), cold 1% OsO_4 at pH 6.0, 1% uranyl acetate block staining and Araldite 506 embedding. Sections were stained by the permanganate-lead sequence^{6,8}. In rigor MYAC layers, pairs of angled cross-bridges form chevrons pointing towards the M line. Nested lead and rear chevrons (labelled L and R) form a doublet every 38.7 nm, with interdoublet gaps of 11–16 nm and intradoublet gaps of 4–8 nm between lead and rear chevrons. Thus, the 38.7-nm repeat is two-thirds filled, as found also in cross-sections, where four out of six of the thin filaments around each myosin receive cross-bridges in a flared X pattern at each 13–14-nm level. *Lethocerus* thick filaments have a four-stranded structure²⁰ with a 38.7-nm helical repeat, and no sign of the vertebrate 43-nm repeat⁴¹. Thin filaments have exactly 14 subunits per turn in both long-pitch (77.3 nm) strands, giving 38.7 nm as the helix 2-fold repeat⁴. The segment within each 38.7 nm, where subunit azimuth is sterically suited for cross-bridge attachments, is designated the actin target zone⁷. Neighbouring thin filaments are related by a 60° rotation (or equivalent 12.9-nm axial translation), producing a left-hand, two-start helix of actin targets surrounding each thick filament. Attachment of rigor cross-bridges labels this helix heavily; the flared X structures rotate 60° from one level to the next as they follow it. *b*, Optical diffraction pattern of the MYAC layer shows strong, sampled layer lines at axial spacings of 38.7 nm and 19.3 nm (plus a weak meridional at 12.9 nm). Only weak diffraction at 14.5 nm survives as expression of the original relaxed thick filament structure.

double chevron (Fig. 3). The limited axial resolution makes angle measurement imprecise due to the relatively broad contact between cross-bridge and actin filament. Nevertheless the lead bridges appear tilted about 50° to the filament axis, a value consistent with that observed in acto-S-1 reconstructions^{9,14,15}. The rear bridges are markedly different and appear to be angled nearly normal (78°) to the filament axis.

The three-dimensional density map viewed down the filament axis (Fig. 4) reveals the two actin long-pitch strands clearly resolved at the lead chevron and interdoublet gap and through

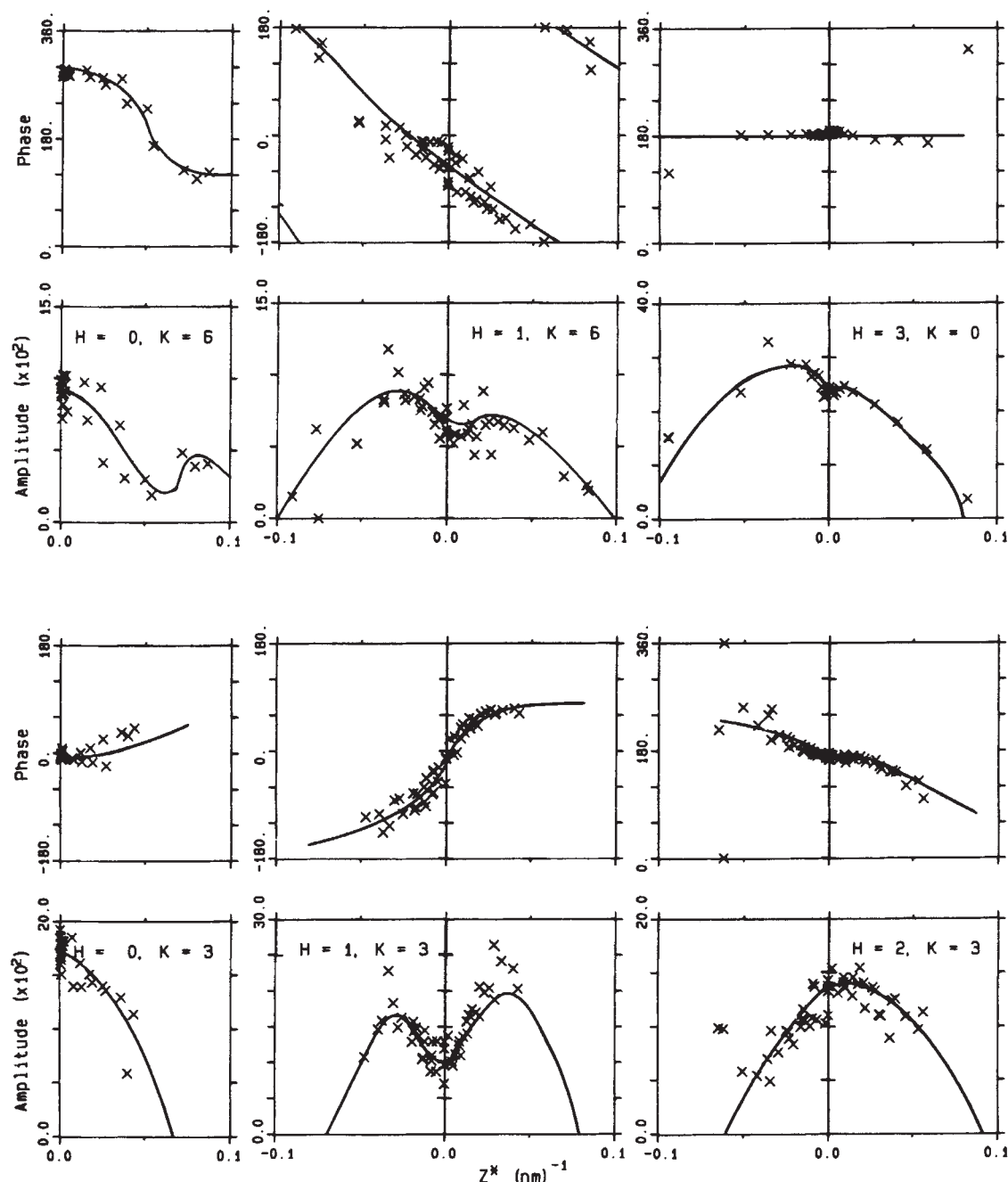


Fig. 2 Amplitude and phase plots for six selected lattice lines in the asymmetric unit for the two-sided plane group p12. Unit cell constants are $a = 46.65$ nm and $b = 116$ nm. Space group selection was based on the fact that, although *Lethocerus* thick filaments have four myosins per 14.5 nm, in rigor the pattern of bridge attachments effectively reduces the rotational symmetry to 2-fold⁷. Random biased rotation of thick filaments³⁶ will not affect their average rotational symmetry, but would cause the points of origin of the bridges on the thick filaments to be smeared azimuthally. At the axial limit of the sampled diffraction (12.9 nm), the molecular transform of the thin filament has even rotational symmetry. Moreover the average symmetry at the actin position may be 2-fold due to random 180° rotation of the thin filaments⁵. Optical diffraction patterns from micrographs of MYAC layers show mirror symmetry about the meridian, further indicating the presence of 2-fold rotation symmetry within the plane of the section. Special care was taken to maintain alignment of the 116-nm period during origin refinement. Smooth curves were drawn through the amplitude and phase plots by hand and sampled at intervals of 0.01-nm^{-1} for purposes of calculating the three-dimensional maps.

about half of the rear chevron region of the map. Thus, we can assign unambiguously the actin position throughout the length of the thin filament; that position confirms the Taylor and Amos model for the cross-bridge-actin binding geometry⁹.

In situ myosin bridges show an important difference from those seen in myosin S-1 in the region distal to the thin filament (Fig. 4). Reconstructions of S-1-decorated actin usually show the portion of the molecule distal to the actin binding site slewing or bending in a single curve around the axis of the thin

filament^{9,14-16}. The rear bridges connect to the surface of the thick filament by following the conventional S-1 slew curvature. On the other hand, the lead bridge bends backwards against the slew observed for S-1 alone. The pronounced departure from acto-S-1 structure occurs some 6-8 nm from the actin binding site.

Lead and rear bridges differ in their overall length by about 1.5 nm (Table 2), a value that probably represents a lower limit because more of the rear bridge length lies nearly parallel to

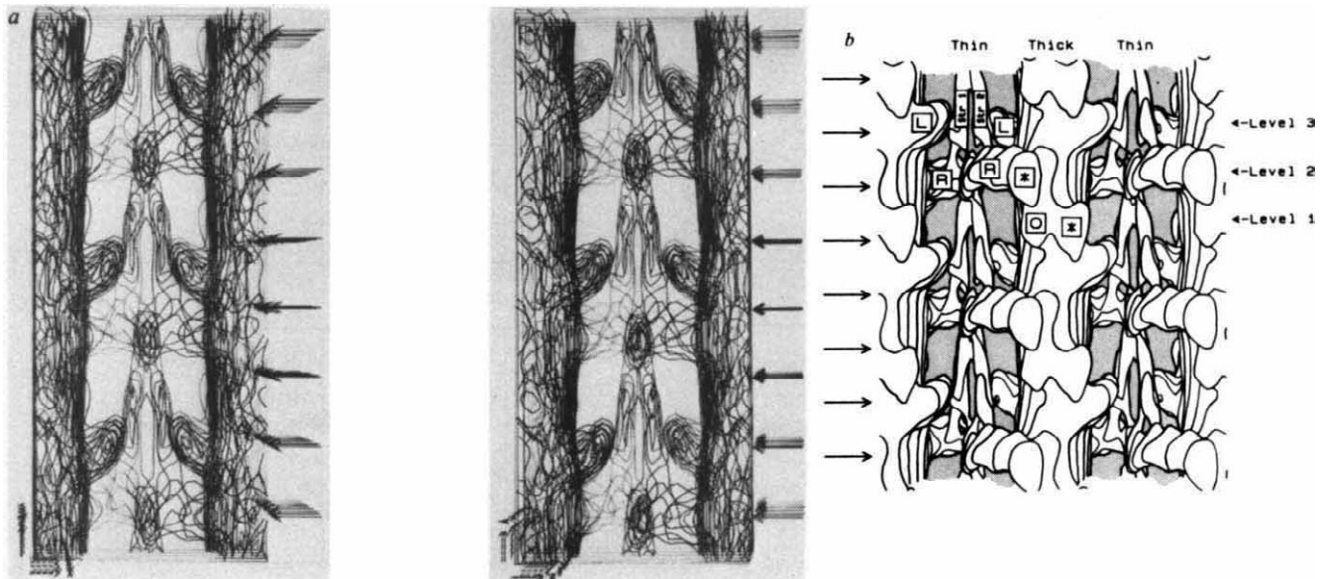


Fig. 3 Three-dimensional density maps viewed perpendicular to the plane of the section. *a*, Stereo pair showing a complete 116-nm repeat. The orientation has the Z-line at the bottom and the M-line at the top. *b*, Same map as shown in *a*, calculated with perspective and with hidden lines removed to illustrate the surface of the structure. Arrows to the right of the figure in *a* and left of the figure in *b* locate the positions of maxima in density contributed by the 14.5-nm meridional reflection, presumably indicating points of regularity in the myosin repeat of the thick filament. L, R, Lead and rear chevrons; *, 0, lead and rear bridges projecting out of the section; Str 1, Str 2, two long-pitch helical strands of the thin filament. Levels 1, 2 and 3 point respectively to the interdoublet gap, the rear and the lead chevrons. A disconnected density at the very surface of the maps in *a* and *b* has been removed for clarity here, but has been retained in Fig. 4. In the maps, protein is represented by the negative densities, due to the fact that in the specimen itself the protein is actually positively stained and shows up in the micrograph transparencies as regions of low optical density. We have chosen, however, to refer to the protein as high density and embedding media as low density (which is in fact the case in the actual specimen). The contour cutoff was chosen by examining the densities that were clearly outside the section and thus either represent noise or Fourier ripple. These noise features for the most part all fell below 8.6% of the highest (negative) density in the map. All contours shown are equally spaced apart. The imposed 2-fold rotation symmetry would effectively smear the bridge density by ± 1.38 nm axially about its centre of mass.

the axis of sectioning compression than that of the lead bridge. These lengths for the cross-bridges are comparable to the 17–19-nm length of myosin heads measured by rotary shadowing¹⁷ and the 17.5-nm length of myosin S-1 when the regulatory class of light chain is present¹⁵. Some of the rear bridges' extra length could be contributed by a part of the myosin subfragment 2 (S-2) portion being drawn away from the thick filament backbone. Because the S-2 region extends vertically upward in Fig. 3, it might be expected that as it is pulled away from the thick filament shaft, the apparent tilt angle will correspondingly be reduced. However, as the amount of S-2 in question is small, this effect cannot fully account for the lower tilt angle; we conclude that the rear bridges are oriented nearly perpendicular to the filament axis. The differences in both slew curvature and tilt angle of the bridges suggest a flexible region within the myosin heads that extends nearly half the distance from their thick filament origin to the actin binding site.

Two myosin heads per cross-bridge

An important current model for rigor insect flight muscle is the Offer and Elliott two-filament-binding model¹⁸. That model is based on the hypothesis that the distortion suffered by myosin when both heads bind to adjacent actin monomers on the same thin filament¹⁹ can be relieved if the two heads bind to separate thin filaments. For a thick filament with four myosin molecules per 14.5-nm level²⁰, and exclusive two-filament-binding for myosin bridge attachments, only 56% of the myosin heads can be attached to actin in rigor insect flight muscle and only 28% of the actin monomers can be labelled²¹. This model supposes that myosin heads not bound to actin are highly disordered or retracted against the thick filament shaft and are therefore not visible in electron micrographs.

The two-filament-binding hypothesis predicts that a MYAC layer should have one non-bridging myosin molecule in the interdoublet gap in an orientation unfavourable to actin binding.

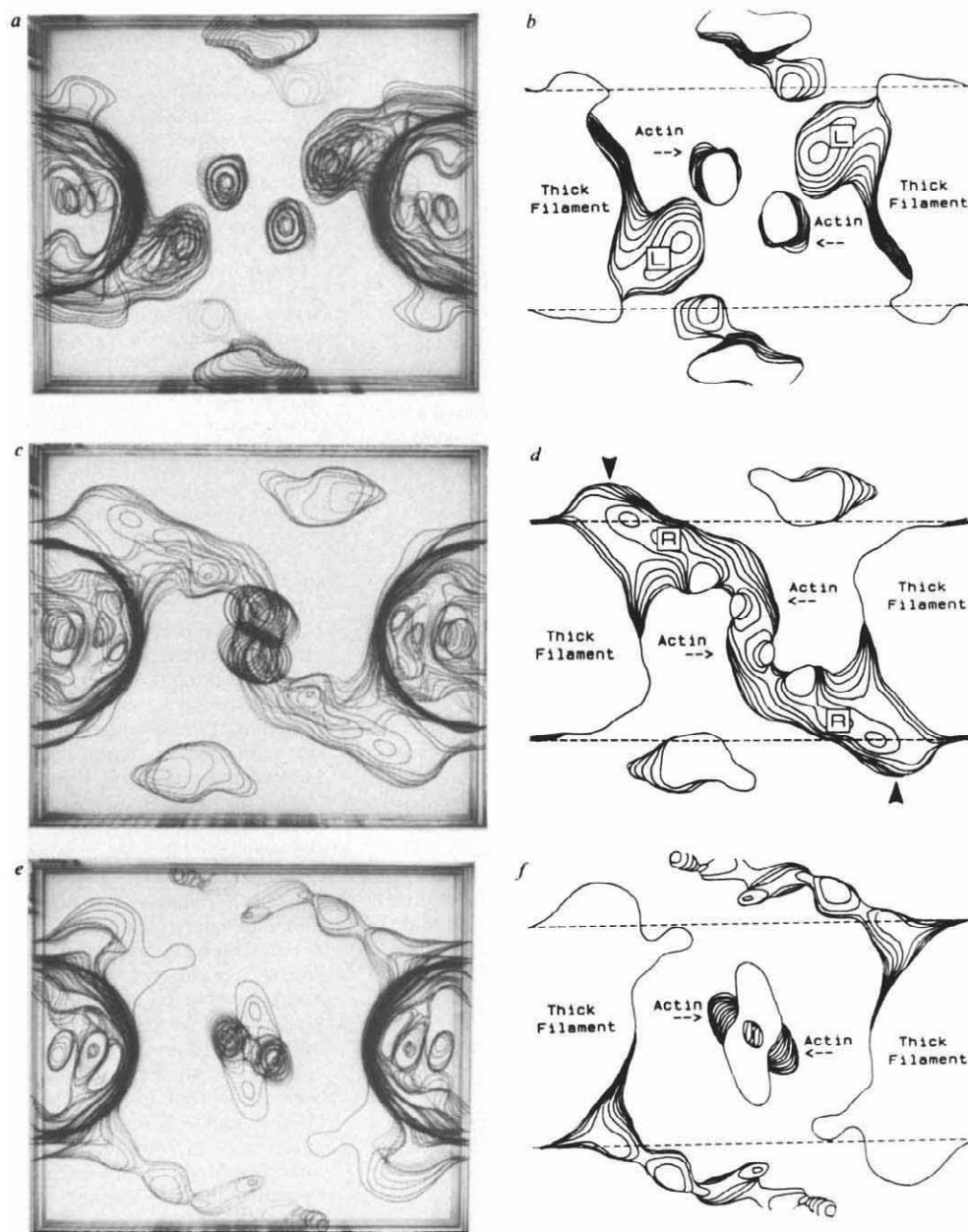
In the reconstruction method we have used, poorly ordered heads would produce a localized, diffuse density on the surface of the thick filament whereas totally disordered heads would raise the average density of the background embedding medium. Our density maps, however, show the filament surface at the interdoublet gap to be extremely smooth (Fig. 3). Moreover, the contour gradient defining the thick filament surface is steepest, and the electron transparency of the embedding medium is greatest, in the MYAC section midplane where the non-bridging myosins should occur. We interpret this to mean that there are no non-bridging myosin molecules in the interdoublet gap, an observation which is not consistent with exclusive two-filament binding for the bridges.

In failing to show evidence for unbound heads, the density map suggests that every myosin molecule is attached to actin. At the same time, the 11-nm axial dimension of the bridges is large enough to contain two myosin heads. However, in rigor insect flight muscle only 70–80% of the myosin heads are attached to actin^{22–24}, which appears to exclude the possibility that all the cross-bridges consist of two heads. A resolution to this problem can be found in the reconstruction. Lead and rear bridges have significantly different density (Table 2). We feel that the bridge density probably reflects the number of bound myosin heads composing it; that is, the lead bridge may have on average two heads while the rear bridge averages a single head. This may be coupled with greater axial disorder in the rear bridges, because a single head may make a random choice of one of two possible actin monomers separated by 5.5 nm. Delocalizing one myosin head over two adjacent actin sites would yield an averaged rear bridge, lower in density but similar in dimensions to the lead bridge.

Thin filament structure

The thin filaments of insect flight muscle differ from those of vertebrate striated muscle in having two actin species, of

Fig. 4 Three-dimensional density maps viewed down the filament axis, looking towards the Z-line. These three maps show a complete 38.7-nm repeat from the bottom third of Fig. 3. *a*, Lead chevron region. Lighter lines represent the protein density. The average thick filament backbone diameter has been indicated by circles drawn with darker lines. *b*, Same map as in *a* but drawn with hidden lines removed and perspective added. Dashed lines outline the region of the map shown in Fig. 3; L, lead chevron; *c*, *d*, rear chevron region; R, rear chevron. Arrows indicate remnants of a lead chevron, directed out-of-plane, which was largely removed in the sectioning process. The averaged actin position, as shown in Fig. 5, has been indicated by 5.0-nm circles drawn with darker lines. *e*, *f*, Interdoublet gap region.



molecular weight 42,000 and 55,000 in a molar ratio of 6:1 (B. Bullard, personal communication), as well as a troponin complex with more than twice the mass of its vertebrate counterpart²⁵. Because our reconstruction approach was restricted to the sampled portion of the diffraction pattern, the actin subunit structure of the thin filament cannot be observed. The long-pitch helical components, however, remain.

The most surprising feature of the thin filament is the change in its helical structure. Expecting two long-pitch helical strands, of fixed separation and constant twist, we find instead these parameters varying dramatically to give two different regions. Near the lead chevron, the strands are nearly untwisted and widely separated (Fig. 5). Conversely, at the rear chevron, the strands are overtwisted but more normally spaced. The twist and separation of strands differ between lead and rear chevrons but remain constant within each region, with changes occurring in the gaps between chevrons. These features of thin filament structure were largely unsuspected from our previous work, although they can be seen in original images once the reconstruction

alerts the observer. A three-dimensional reconstruction of a rigor actin layer (K.A.T. *et al.*, unpublished) confirmed these features and clearly showed the overtwisting of the actin long-pitch helices at the rear chevron.

The contribution of the specimen preparation procedure to these features is of some concern, given the known sensitivity of F-actin to our fixatives^{26,27}. The observed form of the cross-bridges appears validated by both the degree with which the X-ray features of the native muscle are retained⁸, as well as their general similarity to those seen in acto-S-1 reconstructions. Although the variable twist of actin could represent an artefact, it finds reasonable precedent in recent studies of isolated F-actin²⁸. Variable strand separation, however, has as yet no precedent in other work and no recognized correlate in X-ray studies of either native or preserved muscle. Fixative cross-linking between strands might occur more easily in the overtwist zone where the heightened degree of twist tightens the filament, than in the undertwist zone. Strand separation appears more likely to be an artefact than variable twist or cross-bridge shape.

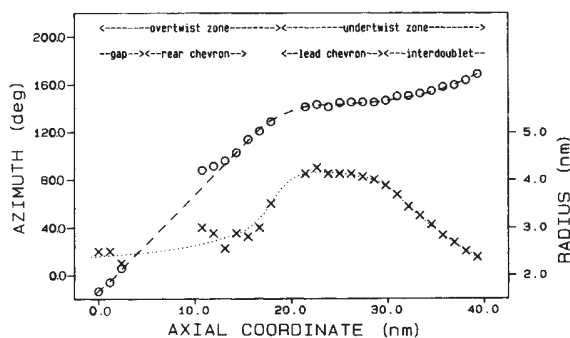


Fig. 5 Plot of the azimuth and centroid radius of the actin long-pitch helices. Axial coordinate is the filament axis. Azimuth is measured relative to the *a* axis (interfilament axis) of the section. Each x, 0 vertical pair represents measurements at one axial position of radius and azimuth respectively. There was ambiguity in assigning the actin density where missing data points occur. The dashed and dotted curves are averaged values drawn in by hand.

The changes in F-actin twist seen here exceed those observed in other systems and may arise because of the stronger interactions produced by pairs of opposed rigor bridges. In particular, untwisting of the thin filament could relieve strain in the myosin due to two-headed binding at the lead chevron. Conversely, overtwisting the thin filament may make two-headed binding at the rear chevron more difficult. Interestingly, actin filaments saturated with untethered heavy meromyosin (HMM), in which most HMM is probably bound with two heads, do not untwist²⁹, probably because the energy required to do so is too great. In this case the distortion is absorbed by the HMM molecules. In the myofibrillar lattice, complete filament saturation is impossible, so changes in actin filament twist in one area can be compensated elsewhere.

The troponin component of the thin filament is not clearly resolved from the myosin bridges. Most attempts to model the X-ray diagram from insect flight muscle^{5,21} and arthropod muscles³⁰ have concluded that the troponin position bears a constant relationship to the bridge marking and makes a significant contribution to diffraction on all layer lines. Given that troponin is distributed on a lattice, it should be resolvable, unless it lies in close proximity to a bridging myosin. The reconstruction suggests, therefore, that troponin does not lie near the lead chevron, or in the interdoublet gap, where it should be easily visible. However, defining its possible relation to the rear chevron must await antibody labelling experiments.

Models for rigor bridge distribution

The reconstruction as we interpret it features lead bridges containing two myosin heads and rear bridges containing a single myosin head, with all myosin molecules bound to thin filaments. Myosin molecules bound to a single thin filament predominate. This interpretation has important consequences for the distribution of bridges within the myofilament lattice, and particularly for the predicted 116-nm repeat. The presence of lead and rear chevrons in each target zone in rigor, and the observed change in thin filament structure, implies two distinctive regions within each target. We will refer to lead and rear bridge targets separately, recognizing that they are subtargets within each of Reedy's original target zones⁷.

Surrounding each thick filament are 36 actin subtargets in each 116-nm period, divided equally between those marked by lead bridges and those marked by rear bridges. In that same 116-nm period there are only 32 myosin molecules, or 64 myosin heads. During the transition from resting to rigor states, it is plausible that some actin subtargets are more accessible to myosin and form rigor bridges more readily than others. Our three-dimensional reconstruction suggests that lead bridge targets are probably always occupied by myosin molecules,

Table 2 Comparison of lead and rear bridges

	Lead	Rear
Length overall	16.0 nm	17.5 nm
Radial component	14.8 nm	~17.5 nm
Axial component	6.0 nm	—
Tilt angle	50°	78°
Maximum density	1.24	0.70
Bound myosin heads	2	1

Lengths were determined by measuring the distance from the point of origin of the bridge on the thick filament shaft along the main body of the bridge to the actin binding site. The surface of the thick filament was a circle of 10 nm radius while the actin position used was the averaged one given in Fig. 5. Bridge tilt angle was measured along the axis of the bridge from the density map calculated perpendicular to the thick filament axis (Fig. 3) and represents only the component projected onto the plane of the section. Densities are expressed as a percentage of the maximum thick filament backbone density at the same distance from the central plane of the reconstruction. This was done to compensate for any variations in contrast that might occur with increasing distance from the central plane of the reconstruction. Note that the lead bridge has nearly twice the density of the rear bridge.

either because their geometry is more favourable to initial bridge formation, or because after the rigor lattice anneals to equilibrium they represent a less strained and lower energy configuration. In forming the rigor lattice, 18 of the 32 myosins will form lead bridges, essentially all containing two myosin heads. The remaining 14 myosin molecules occupy all but 4 of the remaining 18 rear bridge targets. Because of the overtwisting of the actin filament at the rear bridge targets, rear bridges can have no more than one head bound.

This arrangement makes the following predictions. First, 50 of the available 64 myosin heads are attached (78%) and 50 of the available 126 actin monomers are labelled (40%). Second, in some MYAC layers, rear bridges are missing. As these missing rear bridges are likely to be symmetrically disposed about any thin filament, two out of the three MYAC layers which intersect at each thick filament would have a missing rear chevron. The arrangement also predicts that two of the nine levels of flared X structures in the 116-nm period would have two arms missing. Under this scheme, the 116-nm period would arise due to the incomplete occupancy of rear bridge targets.

However, no such systematic absences of rear bridges have been recognized in MYAC layers or flared X cross-sections (M.C.R. and M.K.R., unpublished). In order to produce a flared X on every 12.9-nm level, and occupy all rear bridge targets, some two-filament binding must occur. Within the framework described above, occupancy of all 36 actin subtargets could be accomplished if 4 of the 32 myosin molecules bind to two thin filaments. Such an arrangement need not increase the total number of bound myosin heads, as the additional rear bridges would come about by 'rearranging' the second bound head of a lead bridge. In this case, the 116-nm period would arise due to the different degrees of head occupancy of the actin subtargets. An experimental test between these two models can be made based on the observed frequency of missing rear bridges both in MYAC layers and in flared X structures.

Both of these arrangements produce a variation in bridge density because rear bridge targets never have more than a single myosin head bound (but may on occasion have none) and lead bridge targets never have less than a single head bound (but most of the time have two). This latter statement supplies specific occupancy details for the spatial concentration function (*Q* function), which is used to describe cross-bridge binding probability along actin in calculations of the rigor X-ray diagram of insect flight muscle⁵.

Discussion

Our reconstruction of the rigor insect flight muscle MYAC layer has two features of importance to muscle contraction. One is

the demonstration of two different rigor cross-bridge structures, confirming our recent observations⁸. The differences in the two types of averaged rigor bridges imply a rather high flexibility with respect to both azimuth and tilt in the myosin heads. Of equal importance is the twist variation of the thin filament. Azimuthal flexibility of myosin heads is a feature of the detailed mechanism proposed for muscle contraction³¹, while twist variation in F-actin filaments was an early observation³² that has recently been described in detail²⁸.

Perhaps more surprising than the flexibility shown in the myosin heads is the finding that it expresses itself in two averaged conformations. We have become accustomed, due to three-dimensional reconstructions of acto-S-1^{9,14-16} as well as electron paramagnetic resonance studies³³, to a picture of rigor bridges in which all myosin heads show a uniform structure. In the myofilament lattice, however, rigor bridges tethered to the thick filament are not free to achieve a uniform orientation.

Our reconstruction suggests strongly the presence of more degrees of freedom than is often supposed in the formation of the rigor cross-bridge lattice. Some constraints must operate, otherwise no ordered lattice would be produced. However, the mismatch between axial periods of thick and thin filaments itself does not appear to prevent myosins at any axial level from forming cross-bridges, an interpretation also supported by the uniform 12.9-nm repeat in images of freeze-fractured, deep-etched muscle³⁴. Thus, in rigor insect flight muscle, all myosins appear able to reach at least one actin target to form an ordered lattice of lead and rear chevrons as well as flared X structures. This would be remarkable enough with all the thick filaments in rotational register; it is even more remarkable if it occurs in the presence of a random rotational disorder of the thick filaments³⁵.

We have proposed two related models for the rigor cross-bridge lattice, one based on myosin molecules binding exclusively to a single thin filament and a variation which includes some myosins binding to two thin filaments. Both models draw support from measurements of the fraction of bound myosin heads²²⁻²⁴, as well as the fraction of actin molecules bound to myosin²². A consequence of exclusive single-filament binding of myosin heads is the lack of cross-bridge binding to some actin subtargets. Myosin head occupancy of all actin targets can be achieved with only four of the available myosins (12%) taking part in two-filament interactions. Recent work³⁶ has sug-

gested that some two-filament interactions can occur in rigor myofibrils, but leaves open the question of their relative frequency. Freundlich and Squire³⁵ have proposed that mixed two-filament and single-filament interactions occur in insect flight muscle in rigor. Our model differs from theirs in the important aspect of assigning specific degrees of head occupancy to the separate lead and rear bridges, as well as setting an upper limit on the frequency of two-filament interactions, should they occur.

The different shapes of the lead and rear bridges may reflect not only their geometrical situation but a temporal attachment sequence as well. Rigor stiffness and tension take 20–90 s to develop in single fibres³⁷. Thus attachment may occur earlier for some bridges and later for others. We can speculate that the early and late phases may be systematically distributed between lead and rear bridges. For example, the more angled lead bridges may represent the first group of myosin heads to attach. The rear bridges attach later and are ultimately less tilted, either because they are unable to execute a full power stroke against the resistance of the lead bridges, or because they rotate fully and drive the lead bridges to a steeper angle.

Major features of the reconstruction may also relate to steps in the active cross-bridge cycle. Recent experiments with phalloidin-labelled actin have been interpreted in terms of changes in the structure of the actin molecule during contraction³⁸. X-ray diffraction patterns of active muscle have indicated that the cross-bridges do not adopt the symmetry of the actin filament during contraction. Huxley suggested that this was due to azimuthal disorder of attached cross-bridges around the thin filament and/or azimuthal disorder of the actin helix itself³⁹. Both the twist variation in thin filaments and the two forms of cross-bridges seen here in an ordered lattice could contribute to the azimuthal disorder postulated by Huxley if they were irregularly distributed in actively contracting muscle. Thus rigor cross-bridges distributed in a lattice may illustrate important features of the power stroke that drives muscle contraction.

We thank the MRC Laboratory of Molecular Biology for use of their scanning microdensitometer, Mrs Pat Thompson and Mrs Belinda Irsula for typing this manuscript, Mr Alan Farmer for technical assistance, and especially Dr Hugh Huxley for his comments on the manuscript. This research was supported by grants NIH GM 30598, AM 14317 and grants from the Muscular Dystrophy Association.

Received 16 March; accepted 3 July 1984.

1. Reedy, M. K., Holmes, K. C. & Tregear, R. T. *Nature* **207**, 1276–1280 (1965).
2. Reedy, M. K. & Garrett, W. E. in *Insect Flight Muscle* (ed. Tregear, R. T.) 115–136 (Elsevier, Amsterdam, 1977).
3. Reedy, M. C., Reedy, M. K. & Goody, R. S. *J. Muscle Res. Cell Motility* **4**, 55–81 (1983).
4. Miller, A. & Tregear, R. T. *J. molec. Biol.* **70**, 85–104 (1972).
5. Holmes, K. C., Tregear, R. T. & Barrington Leigh, J. *Proc. R. Soc. B* **207**, 13–33 (1980).
6. Reedy, M. K., Goody, R. S., Hofmann, W. & Rosenbaum, G. *J. Muscle Res. Cell Motility* **4**, 25–53 (1983).
7. Reedy, M. K. *J. molec. Biol.* **31**, 155–176 (1968).
8. Reedy, M. K. & Reedy, M. C. *J. molec. Biol.* (submitted).
9. Taylor, K. A. & Amos, L. A. *J. molec. Biol.* **147**, 297–324 (1981).
10. Henderson, R. & Unwin, P. N. T. *Nature* **257**, 28–32 (1975).
11. Akey, C. W. & Edelstein, J. *J. molec. Biol.* **163**, 575–612 (1983).
12. Wray, J. S. *Nature* **280**, 325–326 (1979).
13. Squire, J. M. *J. molec. Biol.* **77**, 291–323 (1973).
14. Moore, P. B., Huxley, H. E. & DeRosier, D. J. *J. molec. Biol.* **50**, 279–295 (1970).
15. Vibert, P. & Craig, R. *J. molec. Biol.* **157**, 299–319 (1982).
16. Toyoshima, C. & Wakabayashi, T. *J. Biochem.* **86**, 1887–1890 (1979).
17. Elliott, A. & Offer, G. *J. molec. Biol.* **123**, 505–519 (1978).
18. Offer, G. & Elliott, A. *Nature* **271**, 325–329 (1978).
19. Greene, L. E. & Eisenberg, E. *J. biol. Chem.* **255**, 549–554 (1980).
20. Reedy, M. K., Leonard, K., Freeman, R. & Arad, T. *J. Muscle Res. Cell Motility* **2**, 45–64 (1981).
21. Offer, G., Couch, J., O'Brien, E. & Elliott, A. *J. molec. Biol.* **151**, 663–702 (1981).
22. Goody, R. S., Reedy, M. C., Hofmann, W., Holmes, K. C. & Reedy, M. K. *Biophys. J.* (in the press).
23. Lovell, S. J., Knight, P. J. & Harrington, W. F. *Nature* **293**, 664–666 (1981).
24. Thomas, D. D., Cooke, R. & Barnett, V. A. *J. Muscle Res. Cell Motility* **4**, 367–378 (1983).
25. Bullard, B. *J. Muscle Res. Cell Motility* **1**, 194–195 (1980); **5**, 196 (1984).
26. Lehrer, S. S. *J. Cell Biol.* **90**, 459–466 (1981).
27. Maupin-Szamier, P. & Pollard, T. D. *J. Cell Biol.* **77**, 837–852 (1978).
28. Egelman, E. H., Francis, N. & DeRosier, D. J. *Nature* **298**, 131–135 (1982).
29. Katayama, E. & Wakabayashi, T. *J. Biochem.* **90**, 703–714 (1981).
30. Wray, J., Vibert, P. & Cohen, C. *J. molec. Biol.* **124**, 501–521 (1978).
31. Huxley, H. E. *Science* **164**, 1356–1366 (1969).
32. Hanson, J. *Nature* **213**, 353–356 (1967).
33. Thomas, D. D. & Cooke, R. *Biophys. J.* **32**, 891–906 (1980).
34. Heuser, J. E. *J. molec. Biol.* **169**, 123–154 (1983).
35. Freundlich, A. & Squire, J. M. *J. molec. Biol.* **169**, 439–453 (1983).
36. Borejdo, J. & Oplatka, A. *Nature* **291**, 322–323 (1981).
37. White, D. C. S. *J. Physiol., Lond.* **208**, 583–605 (1970).
38. Prochniewicz-Nakayama, E., Yanagida, T. & Oosawa, F. *J. Cell Biol.* **97**, 1663–1667 (1983).
39. Huxley, H. E., Faruqi, A. R., Kress, M., Borda, J. & Koch, M. H. J. *J. molec. Biol.* **158**, 637–684 (1982).
40. Bennett, P. M. *J. Cell Sci.* **15**, 693–701 (1974).
41. Wray, J. S. *Nature* **277**, 37–30 (1979).

Linearization of maize mitochondrial chromosomes by recombination with linear episomes

Christopher L. Schardl & David M. Lonsdale

Cytogenetics Department, Plant Breeding Institute, Cambridge CB2 2LQ, UK

Daryl R. Pring & Kathryn R. Rose

USDA-ARS Department of Plant Pathology, University of Florida, Gainesville, Florida 32611, USA

Cytoplasmic male sterility in maize is determined by the mitochondrial genotype. One strain of cytoplasmically male sterile maize contains, in addition to the usual circular mitochondrial chromosomes, two short linear episomes, S-1 and S-2. We report here that S-1 and S-2 can recombine with homologous sequences present on the circular chromosomes to convert a high proportion of the mitochondrial DNA into linear molecules with S-1 and S-2 covalently linked to one end.

THE mitochondrial genomes of normal fertile *Zea mays* (maize)^{1,2} and *Brassica campestris* (turnip)³, like those of animals and most fungal species⁴, consist principally of circular DNA molecules. In both of these plant species, the mitochondrial genomes undergo rearrangement due to recombination between repeated sequences, resulting in a variety of circular mitochondrial chromosomes¹⁻³. Maize and *Brassica* also possess small mitochondrial plasmids or episomes, some of which are circular DNA species whereas others, like the maize S-1 and S-2 episomes^{5,6} and the 11.3-kilobase (kb) plasmid of *Brassica*⁷, are linear. However, linear mitochondrial chromosomes, such as those present in some ciliates and the yeast *Hansenula mrakii*⁴, have not been reported in higher plants.

The four maize cytoplasmic types identified to date differ in the sequence organization of their mitochondrial genomes. Three of these cytoplasmic types, designated S (USDA), T (Texas) and C (Charrua), cause pollen abortion resulting in male sterility. The male-sterile cytoplasmic types are distinguished by their patterns of fertility restoration by nuclear restorer genes^{8,9}. The N (normal) cytoplasm allows normal pollen development, even in the absence of known restorer genes⁸. The maize cytoplasmic types can also be unambiguously distinguished on the basis of their mitochondrial polypeptides¹⁰ and the restriction profiles of their mitochondrial DNA (mtDNA)¹¹. In contrast, restriction endonuclease analysis of their chloroplast DNA does not reveal any major differences between the four cytoplasmic types¹¹. Mutants of the S-type and T-type cytoplasmic types that confer fertility on unrestored nuclear backgrounds are associated with specific alterations in the mtDNA¹²⁻¹⁵. These observations indicate that the mitochondrial genome, rather than the chloroplast genome, is the cytoplasmic determinant of male fertility in maize.

The presence of two linear episomes, S-1 (6.4 kb) and S-2 (5.4 kb), characterizes the S-type mitochondrial genomes⁶. These episomes have similar or identical terminal inverted repeats of 208 base pairs (bp)¹⁶⁻¹⁸. The terminal 9-bp sequence of the terminal inverted repeats shows a high degree of homology to the terminal sequences of the *Bacillus* phages ϕ 15, ϕ 29, Nf, M2Y and GA-1 (ref. 18). Like these *Bacillus* phages and adenoviruses, the 5' termini of S-1 and S-2 are covalently linked to a protein¹⁹, suggesting that this terminal complex may function in the initiation of DNA replication.

DNA sequence homology to S-1 and S-2 has been demonstrated in the high-molecular weight mtDNA (mitochondrial chromosomes) from the N-type and S-type cytoplasmic types^{1,12,14,20}, but seems to be virtually absent from the C-type and T-type cytoplasmic types²¹. In S-type cytoplasmic types that have reverted to fertile forms, the free S-1 and S-2 episomes are no longer detectable^{12,15}. Restriction enzyme analysis of the mtDNA from these revertant cytoplasmic types also reveals discrete alterations associated with sequences in the mitochondrial chromosomal DNA that are homologous to S-2 (refs 12, 14). It has been suggested that these changes result from either the integration of S-2 or the rearrange-

ment and amplification of the S-2-homologous sequences already present in the mitochondrial chromosomes^{12,14}. In either situation the implication is that the rearrangements of S-episome sequences are involved in fertility reversion of the S-type cytoplasmic types.

To define the specific structural changes involved in reversion of S-type cytoplasmic types to fertile forms, it is necessary to know the extent and location of the sequences homologous to S-1 and S-2 which are present in the mitochondrial chromosomes of the parental male-sterile cytoplasmic types. We show here the surprising result that, in the presence of the S-episomes, much of the mtDNA is in the form of linear mitochondrial chromosomes with copies of S-1 and S-2 residing at the ends of the linear molecules.

Comparison of S and VG mtDNA

The two male-sterile S-type cytoplasmic types investigated, designated S and VG (ref. 15), differ in the nuclear genotypes with which they are associated. The nuclear background of S is Wf9, whereas VG is an S-type cytoplasm in an M825 nuclear background. Neither nuclear genotype possesses S restorer genes and therefore both exhibit the male-sterile phenotype. However, the M825 nuclear genotype of VG confers a high reversion frequency on the VG cytoplasm (up to 11% fertile plants and plants with fertile sectors), in contrast to Wf9(S) in which reversion is rare¹⁵. In addition, the relative proportions of S-1 and S-2 are under nuclear gene control¹⁵.

To investigate the regions of the mitochondrial chromosomes containing homology to the S-episomes, mtDNA from S and VG was freed of S-1 and S-2 by isopycnic and rate zonal centrifugation and subjected to restriction analysis. As the episomes were almost undetectable in these preparations (Fig. 1, tracks 4, 5), the S-episome sequence homologies in the restriction profiles of high-molecular weight mtDNA from S and VG (Fig. 1, tracks 6, 7) represented sequences in the mitochondrial chromosomes. These *Bam*HI restriction profiles, when probed with S-episomes, showed similar hybridization patterns, indicating that the mtDNAs in the S and VG cytoplasmic types are similarly organized with respect to the S-episome-homologous sequences (Fig. 1, tracks 6, 7).

S-1 and S-2 homologous sequences

S-1 and S-2 sequences in the mitochondrial chromosomes were analysed by cosmid mapping. Approximately 1,250 cosmids were prepared by the method described previously^{20,22}. These cosmids contained cloned mtDNA from S-type cytoplasmic types (500 cosmids from VG and 750 from S). Several cosmids contained complete or nearly complete copies of the S-episome sequences (Figs 2, 3). Of these, two (CSB 488 and CSB 481) contained complete S-1 sequences and one (CSB 316) contained a complete S-2 sequence. Restriction endonuclease analysis of these cos-

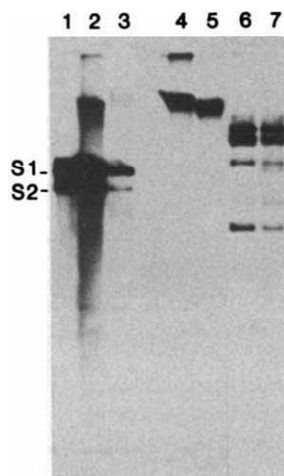


Fig. 1 S-episome homology in maize high-molecular weight mtDNA from the S-type cytoplasms. Uncleaved mtDNA preparations were purified S-1 and S-2 (lane 1), total S mtDNA (lane 2), total VG mtDNA (lane 3) and preparations of high-molecular weight mtDNA from S (lane 4) and VG (lane 5). High-molecular weight mtDNA preparations cleaved with *Bam*HI restriction endonuclease were from S (lane 6) and VG (lane 7).

Methods: Mitochondrial DNA was prepared from etiolated maize shoots as described previously^{20,26}. High-molecular weight and S-episome fractions were prepared by isopycnic and rate-zonal ultracentrifugation in CsCl density gradients. Restriction endonuclease digests were as recommended by BRL. DNA samples were size-fractionated by electrophoresis in a 1% agarose gel, blotted onto a nitrocellulose filter²⁷ and probed by hybridization to nick-translated²⁸ S-episomes.

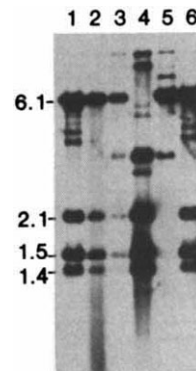


Fig. 2 The extent of S-1- and S-2-episome homology in S and VG mtDNA, and in cosmid clones of S mtDNA, demonstrated by *Pst*I restriction endonuclease analysis. Lanes contain S-1 and S-2 episomes (lanes 1, 6), high-molecular weight mtDNA preparations from S (lane 2) and VG (lane 3) and the cosmid clones CSB 316 (lane 4) and CSB 488 (lane 5). Fragment sizes are in kilobases.

Methods: Cosmid clones were prepared from S mtDNA partially cleaved with *Sau*3A restriction endonuclease. Fragments were size-fractionated by rate-zonal centrifugation on CsCl density gradients ($\rho = 1.19\text{--}1.49$). Fragments of 32–50 kb were cloned into the *Bam*HI site of the cosmid vector pHC79 (ref. 29), as described previously²². The clones were introduced into *Escherichia coli* ED 8767 by transfection, and the Amp^rTc^r transfectants were selected, grown and screened for S-episome homology²². The sizes of the S-episome-homologous *Pst*I fragments were determined from ethidium bromide-stained agarose gels, using *Hind*III-cleaved λ phage DNA and *Taq*I-cleaved pBR322 as molecular size markers.

mid demonstrated that the cloned S-episome sequences included part or all of the terminal inverted repeats. Sites of *Pst*I endonuclease cleavage are located within the terminal inverted repeats, 134 bp from each end of S-1 and S-2 (ref. 18), so that cleavage of the S-episomes with *Pst*I generates internal fragments of 6.1 kb from S-1, and 2.1 kb, 1.4 kb and 1.5 kb from S-2. These four fragments were visualized by *Pst*I restriction analysis of the cosmid clones, and by similar analysis of S and VG mitochondrial chromosomes (Fig. 2). Therefore, not less than 95% of the S-1 and S-2 sequences were present in the integrated copies in the mitochondrial chromosomes of both S-type cytoplasms.

S-episome sequences flanking S-1 and S-2 copies

Only two chromosomal regions, designated σ and ψ , flanked the S-1 and S-2 sequences in the cosmid clones (Fig. 3). The orientation of σ and ψ always corresponded to the orientation of the adjacent terminal inverted repeat sequences. For example, restriction mapping of cosmid CSB 316 revealed that it contained an S-2 sequence flanked on both sides by σ . Like the terminal inverted repeats, the flanking σ sequences were in inverted orientation. Therefore, the S-1 and S-2 sequences could be in either orientation relative to σ and ψ , but the orientation of σ and ψ relative to the attached S-episomes was invariant.

The sequences σ and ψ were also present in cosmids such as VG 3D5, VG 5F10, CSA 51, CSA 61 and CSA 83 that contained minor sequence homology to the episomes (Fig. 3c). The sequences adjoining σ and ψ in these cosmids were designated σ' and ψ' . The minor S-episome homology consisted of the terminal inverted repeat sequence at the σ – σ' and ψ – ψ' junctions. Thus, σ and ψ were found adjoining a variety of sequences: σ' , ψ' , the left-hand ends of S-1 and S-2, and the right-hand ends of S-1 and S-2. Furthermore, the terminal inverted repeat sequence was present at each of these junctions.

In restriction digests of high-molecular weight mtDNA from S and VG, fragments containing the S-1 and S-2 junctions with σ and ψ were always present in lower stoichiometric amounts than the other fragments of mtDNA. Similarly, the σ – σ' and

ψ – ψ' junction fragments were also present in relatively low amounts (data not shown). These data suggested that recombinations between terminal inverted repeat sequences generated the variety of junctions with S-episome homology which were observed in the mtDNA of S-type cytoplasms. Recombination between the circular mitochondrial chromosome and free S-1 and S-2 would result in a linearization of the mitochondrial chromosome (Fig. 4). Evidence for such linearized chromosomes is demonstrated below.

Linear mitochondrial chromosomes

S-1 and S-2 located at the ends of linear chromosomes (Fig. 4) were distinguished from the internally located sequences by restriction analysis. In restriction digests of S and VG mitochondrial chromosomes, fragments were detected that were electrophoretically identical to the homologous terminal fragments from the linear S-episomes (Fig. 5). For example, an S-1-homologous fragment of 5.5 kb was generated by *Sal*I cleavage of the S mitochondrial chromosomal DNA (Fig. 5a). This corresponded to the *Sal*I fragment from the right-hand end of the linear S-1 episome (right-hand and left-hand conventions used as in ref. 18 and Fig. 3). Similarly, the 5.4-kb *Xho*I fragment corresponded to the left-hand end fragment of S-1. The S-2-homologous *Bam*HI fragment of 3.9 kb and *Xho*I fragment of 3.2 kb (Fig. 5b) corresponded to fragments from the left-hand and right-hand ends of linear S-2 respectively. These data suggested the presence of linear mitochondrial chromosomes with S-1 and S-2 covalently attached to their ends. Furthermore, S-1 and S-2 could be attached in either orientation, resulting in linear species terminating at the right-hand end of S-1, the right-hand end of S-2, the left-hand end of S-1 or the left-hand end of S-2.

In the S cytoplasm, the presence of the linear mitochondrial chromosomes was confirmed using the exonuclease *Bal*31, which degrades only linear forms of double-stranded DNA. To observe the effect of *Bal*31 treatment on the regions with S-1 and S-2 homology, *Bal*31-treated mtDNA was analysed by cleavage with *Bam*HI or *Sal*I restriction endonuclease (Fig. 6a,

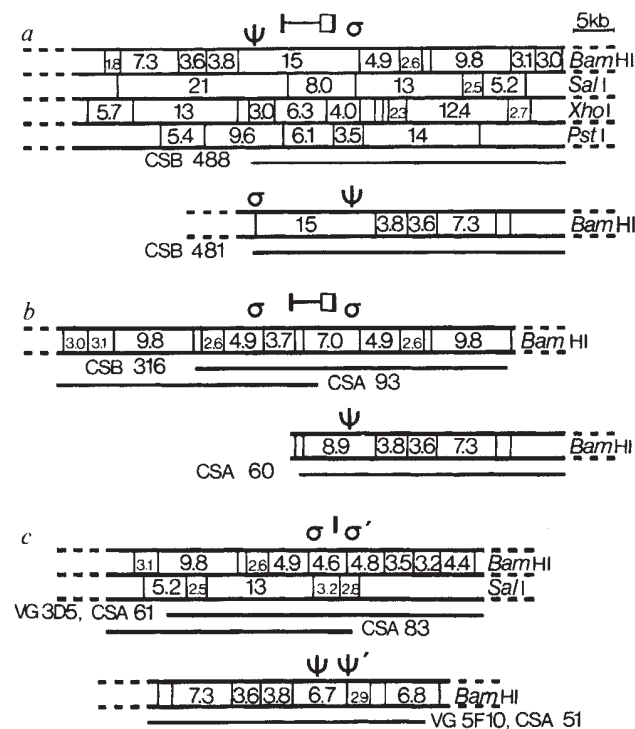


Fig. 3 Restriction endonuclease maps of regions in S and VG mitochondrial chromosomes that contain homology to S-1 (a), S-2 (b) and the terminal inverted repeat sequence of S-1 and S-2 (c). The S-1 and S-2 homologous regions are indicated, and include the terminal inverted repeats (I) and the additional 1.2-kb S-1 and S-2 common sequence (II). Cosmids designated CS and VG contain clones of S and VG mtDNA, respectively.

Methods. Clone pCLS155, used as an S-1 probe, was prepared by ligation of the 1.55-kb *Hind*III fragment from S-1 (ref. 17) into the *Hind*III site of pUC9 (ref. 30). Probes pKRS21 and pKRS15 were the 2.1- and 1.5-kb *Pst*I fragments of S-2, respectively, cloned into pBR322. Cosmid DNA was prepared as described previously²⁰ and mapped by restriction endonuclease cleavage and Southern blot hybridization (see Fig. 1 legend) to other cosmid DNA sequences. Fragments with S-1 and S-2 homology were detected using an S-episome preparation (Fig. 1) as a probe. The S-1 unique sequences were identified by hybridization to pCLS155. The S-2 unique sequences and the 1.2-kb common sequence of S-1 and S-2 were identified by hybridization to pKRS21 and pKRS15, respectively. As pKRS21 and pKRS15 also contain 74 bp of the terminal inverted repeat sequence, hybridization to both probes indicated sites of homology to this sequence at the σ - σ' and ψ - ψ' junctions (c). Fragment sizes were determined as in Fig. 2.

c. *Bam*HI was chosen because it does not cleave S-1, and *Sal*I because it does not cleave S-2 (refs 17, 18). In this way, S-1 and S-2 sequences attached to mitochondrial chromosomes were distinguished from the free episomes. In S mtDNA the predominant regions with S-episome homology were susceptible to *Bal*31 degradation, demonstrating that they were indeed located at the ends of linear molecules.

Like the integrated S-episome sequences (Fig. 3), linear-end forms of S-1 and S-2 were flanked by either σ or ψ (Fig. 6). The major S-1-homologous *Bam*HI fragments were 6.4, 9.7 and 11.7 kb. These corresponded within experimental error to the predicted molecular sizes of free S-1 and the σ -S-1 and ψ -S-1 linear termini, respectively. Similarly, the major S-2-homologous *Sal*I fragments corresponded to free S-2 (5.5 kb), σ -S-2 (8.5 kb) and ψ -S-2 (12.4 kb and >20 kb; the two different terminal *Sal*I fragments from ψ -S-2 arise by a recombination in ψ approximately 5 kb from the left-hand end of the S-episome junction. Recombination also occurs at this position in the N mitochondrial genomes, although S-episome-homologous sequences are not adjacent to ψ in N). Note that the fragments containing internal S-1 and S-2 sequences, namely σ -S- σ , σ -S- ψ , ψ -S- σ

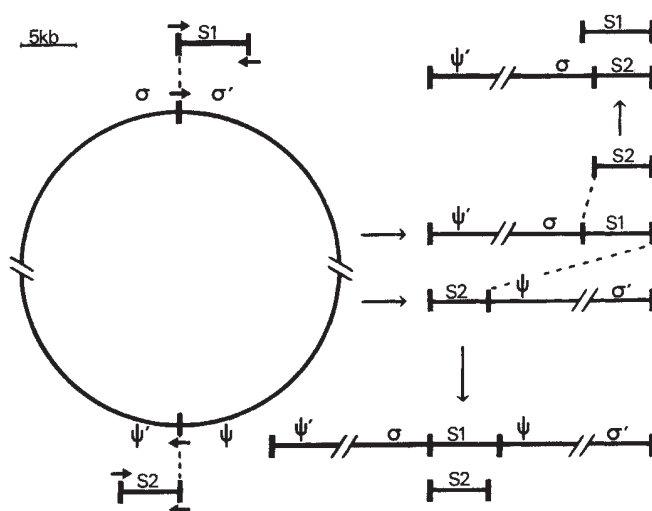


Fig. 4 Model for the linearization of S-type mitochondrial chromosomes and the generation of terminal and internal copies of S-1 and S-2, by recombination between the terminal inverted repeats of S-1 and S-2 (I) and homologous sequences in the mitochondrial chromosomes. Examples of these recombinations are shown. Random and reversible recombinations would result in all of the forms of S-1 and S-2 sequences observed in S and VG mtDNA. These include the eight possible terminal structures σ -S-1, S-1- σ , ψ -S-1, S-1- ψ , σ -S-2, S-2- σ , ψ -S-2 and S-2- ψ , and the eight possible internal structures σ -S-1- σ , σ -S-1- ψ , ψ -S-1- σ , ψ -S-1- ψ , σ -S-2- σ , σ -S-2- ψ , ψ -S-2- σ and ψ -S-2- ψ .

and ψ -S- ψ , were detectable only after long autoradiographic exposure. Although the existence of three internal integrates was demonstrated in the cosmids CSB 481, CSB 488 and CSB 316 (Fig. 3), their stoichiometric amounts in the mtDNA were very low relative to the S-1 and S-2 sequences located at the ends of linear chromosomes.

Linearization of the mitochondrial chromosomes can be postulated (Fig. 4) to arise by recombination of the linear S-episomes with sequences homologous to their terminal inverted repeats, located at the σ - σ' and ψ - ψ' junctions. Alternatively, mitochondrial chromosomes may be linearized by specific cleavage at one end or the other of internally integrated S-episomes. The former model predicts that σ' and ψ' termini are also generated, whereas the latter predicts that the additional products of cleavage are σ and ψ free ends. To distinguish between these possibilities, a fragment containing the σ - σ' junction was used to probe the restriction profiles of *Bal*31-treated mtDNA (Fig. 6b, d). In accordance with the recombination model, a 1.7-kb *Bam*HI fragment and a 0.7-kb *Sal*I fragment were identified. These were the sizes predicted for the fragments derived from the σ' terminus. As expected, these fragments were susceptible to prior *Bal*31 exonuclease digestion. In contrast, the 3.2-kb *Sal*I fragment and the 4.6-kb *Bam*HI fragment, both of which contained σ - σ' (Fig. 3), were internal fragments not susceptible to *Bal*31 exonuclease. Similar results were obtained using a ψ - ψ' probe (data not shown). Furthermore, no prospective σ and ψ free ends were present without attached S-episomes. These results support the proposed recombination model (Fig. 4), as only those termini predicted by this model— σ' , ψ' , σ -S-1, σ -S-2, ψ -S-1 and ψ -S-2—were observed.

The proportion of linear chromosome forms could be estimated by comparing the relative intensities, on autoradiographs, of bands homologous to the σ - σ' junction (Fig. 6b, d). The linear-end fragments (that is, those susceptible to *Bal*31) accounted for over 60% of the total homology in S, with the remainder being internally located sequences. Because there are two sites where linearization can occur (σ - σ' and ψ - ψ'), these results indicate that a high proportion of the mitochondrial chromosomal DNA in S is linear.

Fig. 5 S-1 (a) and S-2 (b) homologous fragments generated by restriction endonuclease cleavage of S high-molecular weight mtDNA (mc) and S-1 and S-2 episomes (ep). a, MtDNA preparations were cleaved with *Bam*HI, *Xho*I or *Sal*I as indicated, size-fractionated by electrophoresis in 1% agarose, blotted²⁷ and hybridized to nick-translated²⁸ S-1-specific probe, pCLS155. The molecular sizes (kb) of the S-1-homologous fragments are indicated. Submolar *Bam*HI fragments that contained the internal S-1 regions σ -S-1- σ (13 kb), σ -S-1- ψ (15 kb), ψ -S-1- σ (15 kb) and ψ -S-1- ψ (17 kb) were visualized only by long exposure. b, The initial probe was allowed to decay and the filter was reprobbed with the S-2 probe pKRS21. Positions of fragments with homology to S-2 are indicated, including the submolar *Sal*I fragments with σ -S-2- σ (11 kb), σ -S-2- ψ (15 kb), ψ -S-2- σ (15 kb) and ψ -S-2- ψ (19 kb).

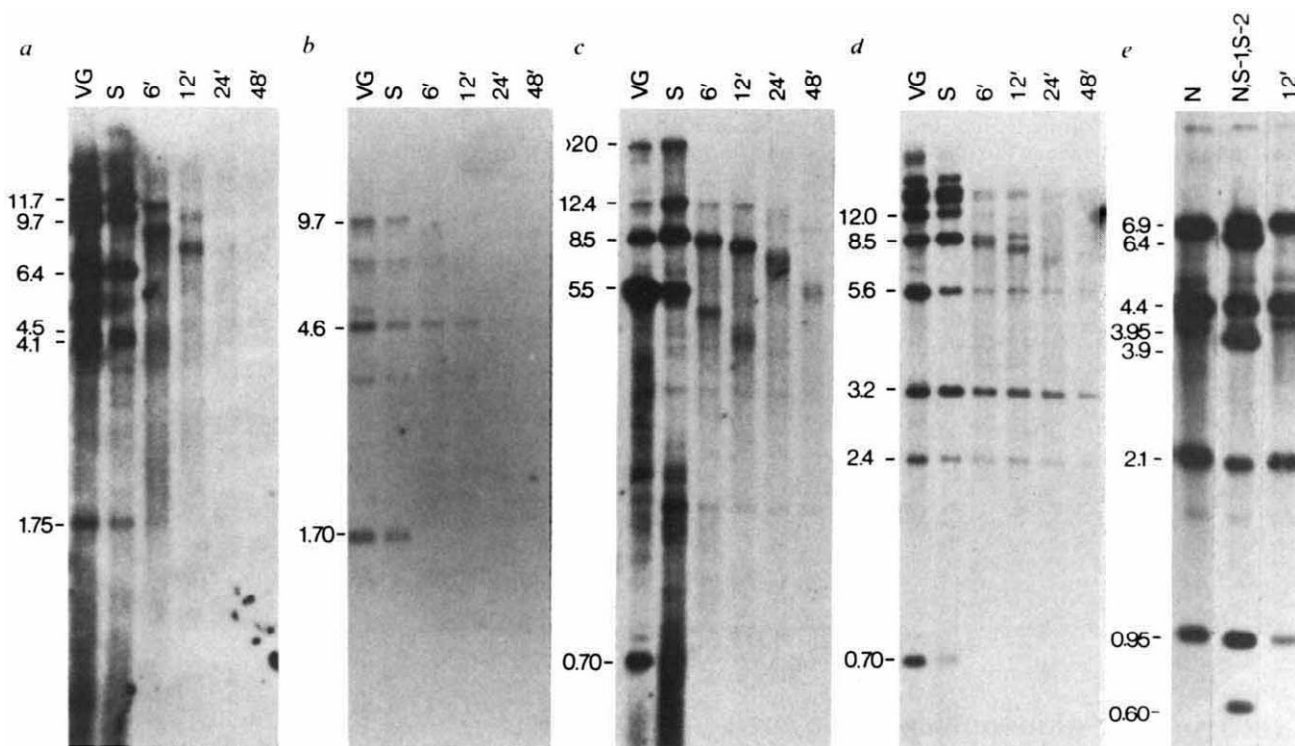
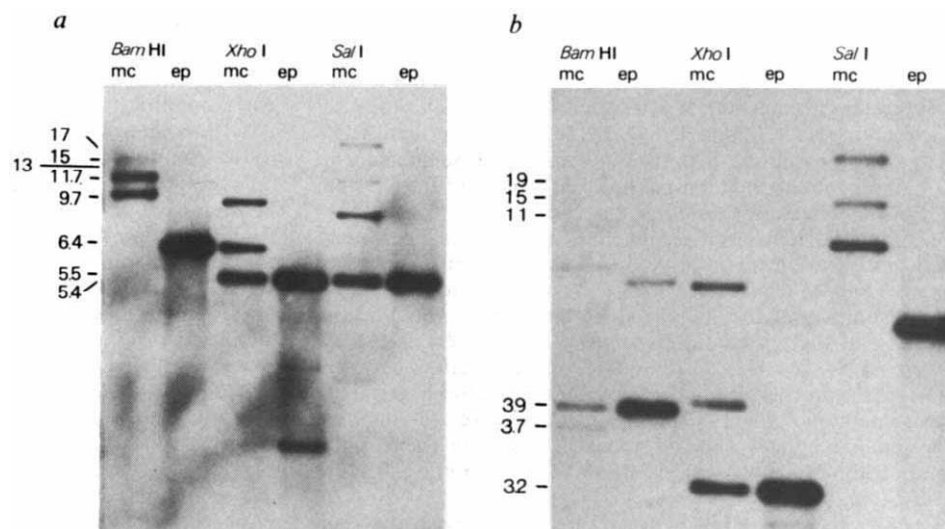


Fig. 6 Demonstration of linear mitochondrial chromosome species with terminal sequences homologous to the S-episomes. a, Untreated VG and S mtDNA is indicated, and S mtDNA treated with *Bal*31 exonuclease (0.5 U per μ g DNA) for the times (min) indicated. mtDNA was subsequently analysed by *Bam*HI cleavage and hybridized to the S-1-specific probe pCLS155. Molecular sizes of the *Bal*31-susceptible fragments are indicated. These correspond to the ends of linear DNA species. Hybridizing fragments smaller than 6.4 kb were specific breakdown products of S-1 episome (unpublished data) and a sequence showing minor homology to S-1 (1.75-kb fragment). b, The filter in a was reprobbed with the 4.6-kb *Bam*HI fragment containing the σ - σ' junction (see Fig. 3). The position of this fragment is indicated, as are the fragments derived from σ' (1.7 kb) and σ -S-1 (9.7 kb) linear ends. Other internal fragments indicate additional regions of homology to σ' . c, Untreated and *Bal*31-treated mtDNA as in a, cleaved with *Sal*I and hybridized to pKRS21, a probe for S-2 sequences. d, The filter in c was reprobbed with the 4.6-kb *Bam*HI fragment containing σ - σ' . The σ' end fragment (0.7 kb) was also visualized in c, due to the 74 bp of terminal inverted repeat sequence in the probe pKRS21. e, An experimental control using, as indicated, untreated N mtDNA, an untreated mixture of N mtDNA, S-1 and S-2 and the same mixture, treated with *Bal*31 for 12 min. mtDNA was subsequently cleaved with *Bam*HI and probed with a mixture of S-1 and S-2. As expected, the S-episome end fragments (6.4, 3.9 and 0.6 kb) were *Bal*31 susceptible, but homologous sequences in N mtDNA were not.

Methods: *Bal*31 treatment was as recommended by BRL. Aliquots containing 0.25 μ g DNA each were phenol-extracted, ethanol-precipitated and subjected to restriction endonuclease cleavage and electrophoresis in a 0.7% agarose gel, then transferred to nitrocellulose and probed with nick-translated DNA. The first probe DNA was removed from the nitrocellulose by the method of Thomas³¹ before reprobbed. Molecular sizes (kb) were determined as in Fig. 2. The 4.6-kb *Bam*HI fragment used as a probe in b and d was isolated from CSA 83 (Fig. 3) cosmid DNA by the method of Yang *et al.*³².

Conclusions

The results presented here lead to a model (Fig. 4) that explains the origins of the various forms of S-1 and S-2 identified in S-mitochondrial chromosomal DNA. It may be postulated that the S-mitochondrial chromosome is circular, as the N mitochondrial master chromosome of 570 kb has recently been shown to be circular (refs 1, 2 and D.M.L., C.M.-R. Fauron and T. P. Hodge, in preparation). In the S-mitochondrial genome, sequences homologous to the terminal inverted repeats of the S-1 and S-2 episomes were identified at the σ - σ' and ψ - ψ' junctions. Recombination between these junctions and the linear episomes would result in linearization and fragmentation of the circular genome. Subsequent recombination events involving the S-1 or S-2 episomes and the linearized chromosomes would cause inversion of the terminal S-episome or replacement of S-1 by S-2 or of S-2 by S-1. Furthermore, recombination between linearized mitochondrial chromosomes containing terminal S-episome-homologous sequences would lead to internal S-episome integrations such as those observed in the cosmid clones CSB 481 (σ -S-1- ψ), CSB 488 (ψ -S-1- σ) and CSB 316 (σ -S-2- σ).

Further, recombination between any two terminal inverted repeat sequences may occur randomly and reversibly. The S-type cytoplasms contain S-1 and S-2 in higher stoichiometric amounts than the mitochondrial chromosomal DNA²¹, so any recombination event between an S-episome and a chromosome is more likely than a recombination between two chromosomal DNA species. As internal S-episome integrations can only arise by recombination events of the latter type, the relative rarity of these species is to be expected. Thus, the S-type mitochondrial genome undergoes a dynamic flux between circular and linear chromosome forms.

Received 8 February; accepted 1 May 1984.

1. Lonsdale, D. M., Hodge, T. P., Fauron, C.M.-R. & Flavell, R. B. *UCLA Symp. molec. cell Biol.*, new Ser. 12, 445-456 (1983).
2. Lonsdale, D. M. *Plant molec. Biol.* (in the press).
3. Palmer, J. D. & Shields, C. R. *Nature* **307**, 437-440 (1984).
4. Wallace, D. C. *Microbiol. Rev.* **46**, 208-240 (1982).
5. Kemble, R. J. & Bedbrook, J. R. *Nature* **284**, 505-506 (1980).
6. Pring, D. R., Levings, C. S. III, Hu, W. W. L. & Timothy, D. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2904-2908 (1977).
7. Palmer, J. D., Shields, C. R., Cohen, D. B. & Orton, T. J. *Nature* **301**, 725-728 (1983).
8. Laughnan, J. R. & Gabay-Laughnan, S. J. *A. Rev. Genet.* **17**, 27-48 (1983).
9. Duvick, D. N. *Adv. Genet.* **13**, 1-56 (1965).
10. Forde, B. G. & Leaver, C. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 418-422 (1980).
11. Pring, D. R. & Levings, C. S. III *Genetics* **89**, 121-136 (1978).
12. Levings, C. S. III *et al. Science* **209**, 1021-1023 (1980).
13. Umbeck, P. F. & Gengenbach, B. G. *Crop Sci.* **23**, 584-588 (1983).
14. Kemble, R. J. & Mans, R. J. *J. molec. appl. Genet.* **2**, 161-171 (1983).
15. Laughnan, J. R., Gabay-Laughnan, S. J. & Carlson, J. E. *Stadler Symp.* **13**, 93-114 (1981).

As noted above, the protein-DNA complexes at the ends of the linear episomes may be involved in episome replication. It is likely that these terminal complexes are also associated with the linearized chromosomes. Therefore, the S-type mitochondrial genome may undergo two alternative replication modes corresponding to the circular and linear chromosome forms. Also, the control of the copy number of linear replicons may differ from that of the circular molecules.

Linearization of the mitochondrial genome occurs in the S-type cytoplasms both in the presence and absence of nuclear fertility restorer genes (data not shown), and may also occur in the fertile Latin American accessions that possess the linear mtDNA species R-1/D-1 and R-2/D-2 (refs 23, 24). Therefore, chromosome linearization alone is not sufficient to cause male sterility. However, cytoplasmic mutation of S and VG to fertile forms results in the loss of S-1 and S-2 as well as the loss of the linear mitochondrial chromosomes²⁵, suggesting a recirculation of the genome. Evidence currently available suggests that this major alteration of the genome structure in the fertile revertants is due to specific mutation of the internally located S-2 sequences²⁵.

The results reported here indicate that the maize mitochondrial genome can exist in many different forms when repeated sequences are available to facilitate homologous recombination, and that in certain maize cytoplasms this process results in an array of linear mtDNA species.

This work was supported in part by Nato grant 283.81 to D.M.L. and D.R.P. C.L.S. is a DeKalb-Pfizer Genetics Corporation fellow. We thank Pioneer Hybrid International, DeKalb-Pfizer Genetics, Dr J. Laughnan and Dr S. Gabay-Laughnan for supplying seed, Tony Hodge for technical assistance and Dr R. B. Flavell for critical reading of this manuscript.

16. Levings, C. S. III, Sederoff, R. R., Hu, W. W. L. & Timothy, D. H. in *Structure and Function of Plant Genomes* (eds Ciferri, O. & Dure, L. III) 363-371 (Plenum, New York, 1983).
17. Kim, B. D., Mans, R. J., Conde, M. F., Pring, D. R. & Levings, C. S. III *Plasmid* **7**, 1-14 (1982).
18. Levings, C. S. III & Sederoff, R. R. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4055-4059 (1983).
19. Kemble, R. J. & Thompson, R. D. *Nucleic Acids Res.* **10**, 8181-8190 (1982).
20. Lonsdale, D. M., Thompson, R. D. & Hodge, T. P. *Nucleic Acids Res.* **9**, 3657-3668 (1981).
21. Thompson, R. D., Kemble, R. J. & Flavell, R. B. *Nucleic Acids Res.* **8**, 1999-2008 (1980).
22. Lonsdale, D. M., Hodge, T. P. & Stoehr, P. J. *Nucleic Acids Res.* **12**, 429-436 (1984).
23. Weissinger, A. K., Timothy, D. H., Levings, C. S. III, Hu, W. W. L. & Goodman, M. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1-5 (1982).
24. Timothy, D. H., Levings, C. S. III, Hu, W. W. L. & Goodman, M. M. *Maydica* **28**, 139-149 (1983).
25. Schardl, C. L., Pring, D. R., Fauron, C. M. F. & Lonsdale, D. M. *Cell* (submitted).
26. Lonsdale, D. M., Hodge, T. P., Howe, C. J. & Stern, D. B. *Cell* **34**, 1007-1014 (1983).
27. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
28. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. J. *molec. Biol.* **113**, 237-251 (1977).
29. Hohn, B. & Collins, J. *Gene* **11**, 291-298 (1980).
30. Vieira, J. & Messing, J. *Gene* **19**, 259-268 (1982).
31. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1980).
32. Yang, R. C.-A., Lis, J. & Wu, R. *Meth. Enzym.* **68**, 176-182 (1979).

LETTERS TO NATURE

Clues from the photonuclear time scale on the nature of particle accelerators in Cygnus X-3 and Vela X-1

R. J. Protheroe

Department of Physics, University of Adelaide, South Australia 5001, Australia

Ultrahigh-energy γ rays ($\geq 10^{15}$ eV) have recently been observed from the binary X-ray sources Cygnus X-3 and Vela X-1. The source of these γ rays remains unknown but it seems likely that protons or nuclei are accelerated, the γ rays resulting from nuclear interactions with matter or radiation. Objects of this type could then contribute significantly to the observed cosmic radiation. The maximum proton energy that can be achieved (this may determine the maximum γ -ray energy) occurs when the acceleration time scale becomes greater than the energy loss time scale, that is, the

attenuation time. Here we have calculated the attenuation times of protons and heavier nuclei in the radiation fields of the stellar companions to the Cyg X-3 and Vela X-1 compact objects. The maximum observed γ -ray energy enables us to put an upper limit on the acceleration time scale of protons at 3×10^{16} eV of 10^4 - 3×10^5 s for Cyg X-3 and 10^5 s for Vela X-1. This result can be used to put some constraints on the nature of the particle accelerator. If binary X-ray sources are contributing significantly to the cosmic rays, the cosmic-ray abundances will be affected by photonuclear interactions.

γ rays of energies above 10^{15} eV have been observed from Cyg X-3^{1,2} and Vela X-1³ and it is likely that they are due to interactions of particles accelerated by the compact objects, possibly pulsars. This has recently been considered for Cyg X-3 by Eichler and Vestrand⁴ who concluded the particles must be protons or nuclei. These particles must have energies in excess of $\sim 10^{16}$ eV to produce the observed γ rays and may contribute significantly to the galactic cosmic ray flux⁵. A flat $\sim E^{-2}$, γ -ray photon spectrum consistent with that observed could imply an $\sim E^{-2}$ particle spectrum consistent with the cosmic-ray source

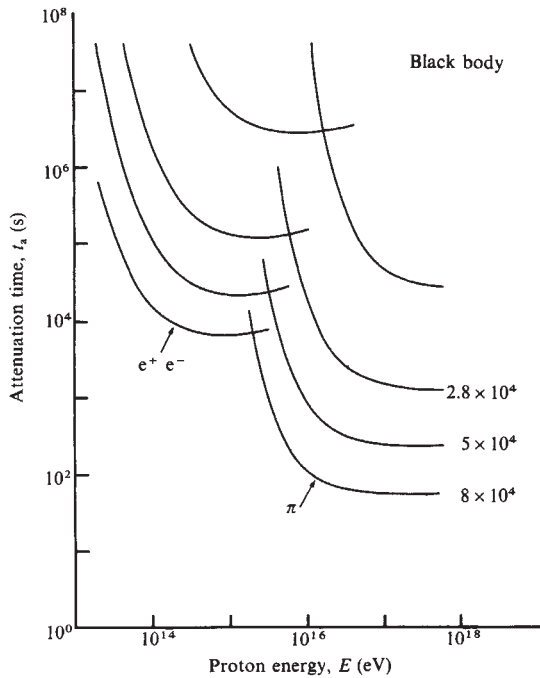


Fig. 1 The attenuation time of protons against hadron production (π) and pair production (e^+e^-) in a black-body radiation field for the temperatures indicated.

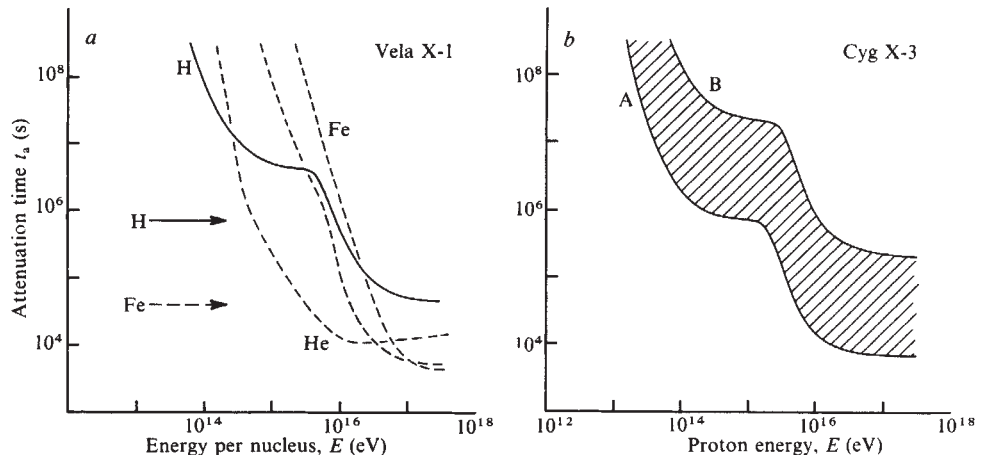
spectrum⁶. To accelerate particles to some energy E , the acceleration time scale must be less than the attenuation time, t_a

$$t_a(E) = E / \left(\frac{dE}{dt} \right) \quad (1)$$

where (dE/dt) is the total rate of energy loss due to various processes.

The starlight photon density will be high in binary X-ray sources due to the presence of a nearby stellar object. The important attenuation processes are then likely to be e^+e^- pair production, hadron production and nuclear photodisintegration. For a particle at rest, the threshold photon energies for these processes are ~ 1 MeV, ~ 0.1 GeV and a few MeV, respectively. The attenuation of a particle of energy $\sim 10^{16}$ eV per nucleon ($\gamma \sim 10^7$) results mainly from photons of energy ~ 0.1 eV, ~ 10 eV and ~ 1 eV for the three processes, that is, the important range of photon energies is in the range emitted as starlight.

Fig. 2 a, The attenuation time against photon interactions of protons and helium, oxygen and iron nuclei in the region of the Vela X-1 compact object. The attenuation times of protons and iron nuclei against interactions with nuclei are indicated by arrows, assuming a matter density of 3×10^9 nuclei cm^{-3} . **b**, The attenuation time of protons in the region of the Cyg X-3 compact object. The hatched area shows the range of possible values between two extreme cases: Case A, separation 1.5×10^{11} cm, stellar mass $2.9 M_\odot$, luminosity 2×10^{37} erg s^{-1} , temperature 8×10^4 K; case B, separation 2.2×10^{11} cm, stellar mass $1.0 M_\odot$, luminosity 9×10^{35} erg s^{-1} , temperature 5×10^4 K. (Separations obtained using 4.8-h orbital period; luminosity and temperature are for a helium star of the given stellar mass and are obtained from ref. 22.)



We will now discuss the attenuation of protons and nuclei by the three processes described above and then consider their application to the attenuation of high-energy particles in Cyg X-3 and Vela X-1. For protons with Lorentz factor γ , the attenuation time against hadron production is given by

$$t_a(\gamma)^{-1} = c \int_0^\infty d\epsilon n(\epsilon) \frac{1}{2\gamma\epsilon} \int_0^{2\gamma\epsilon} k_p(\epsilon') \sigma_{\gamma p}(\epsilon') d\epsilon' \quad (2)$$

where $n(\epsilon)$ is the photon density per unit photon energy, $k_p(\epsilon')$ is the proton inelasticity (average energy lost in the interaction divided by initial energy) on collision of a high-energy proton with a photon of energy ϵ' in the proton rest frame, and $\sigma_{\gamma p}(\epsilon')$ is the total hadron production cross-section. For $\sigma_{\gamma p}$ we used data given in ref. 7 and adopted the form used by Stecker⁸ for $k_p(\epsilon')$. The attenuation time of protons against hadron production in a black-body radiation field is plotted against proton energy in Fig. 1 for a range of temperatures.

The attenuation time of protons against e^+e^- pair production in the 2.7 K microwave background radiation has been calculated by Blumenthal⁹ and this may simply be scaled for attenuation by black-body radiation at a temperature T_e ,

$$t_a(\gamma; T_e) = \left(\frac{2.7 \text{ K}}{T_e} \right)^3 t_a \left(\frac{\gamma T_e}{2.7 \text{ K}}; 2.7 \text{ K} \right) \quad (3)$$

This is plotted against proton energy in Fig. 1 for a range of temperatures. For starlight, the radiation is a diluted black-body radiation and the attenuation time given in equation (3) must be divided by the dilution factor. For nuclei, the attenuation time at a given Lorentz factor is a factor (Z^2/A) lower than for protons.

The mean time for a nucleus of mass A to lose i nucleons by photodisintegration, is given by

$$t_{A,i}(\gamma)^{-1} = c \int_0^\infty d\epsilon n(\epsilon) \frac{1}{2\gamma\epsilon} \int_0^{2\gamma\epsilon} \sigma_{\gamma A,i}(\epsilon') d\epsilon' \quad (4)$$

where $\sigma_{\gamma A,i}(\epsilon')$ is the cross-section for photodisintegration of a nucleus of mass A in which i nucleons are lost; this has been parameterized by Puget *et al.*¹⁰. The attenuation time for photodisintegration of a nucleus of mass A is then given by

$$t_a(\gamma)^{-1} = \sum_i \frac{i}{A} t_{A,i}(\gamma)^{-1} \quad (5)$$

We will now consider the application of the attenuation times discussed above for particles in the radiation field of the stellar objects in Cyg X-3 and Vela X-1. The stellar object¹¹ in the Vela X-1 system is a BO star with apparent magnitude $V \approx 6.9$ at a distance of ~ 1.4 kpc. Assuming a temperature of $T_e \approx 2.8 \times 10^4$ K, we obtain a luminosity $L \approx 8 \times 10^{38}$ erg s^{-1} , after

correcting for interstellar absorption ($A_v = 2.2$). The attenuation times of protons and helium, oxygen and iron nuclei in the radiation field in the region of the compact object at distance¹² of $\sim 3.9 \times 10^{12}$ cm from the centre of the star, are plotted against energy per nucleus in Fig. 2a. In this object, the attenuation time of protons at 3×10^{16} eV is $\sim 10^5$ s—interestingly close to the orbital period of the system ($\sim 8 \times 10^5$ s). This sets an upper limit to the acceleration time at 3×10^{16} eV. Interestingly, if the acceleration time above $\sim 10^{16}$ eV were $\sim 10^4$ s, protons could be accelerated in Vela X-1 above 10^{16} eV but heavier nuclei could not. This contrasts with shock acceleration of cosmic rays, where a rigidity cutoff would result in a deficiency of protons above the cut-off rigidity while Fe nuclei would continue to be accelerated to energies ~ 26 times higher.

If the matter density is high, protons and nuclei will be significantly attenuated by nuclear interactions with the matter. For nuclei of mass A , the attenuation time against fragmentation by nuclei is given by

$$t_a^{-1} \approx nc \sum_i \frac{i}{A} \sigma_{A,i} \quad (6)$$

where n is the number density of nuclei and $\sigma_{A,i}$ is the cross-section for spallation into a nucleus of mass $(A-i)$.

Using the mass loss rate of Vela X-1 obtained by Dupree *et al.*¹³, $\dot{M} \approx 10^{-6} M_\odot \text{ yr}^{-1}$, together with the stellar wind speed of $\sim 860 \text{ km s}^{-1}$ estimated at the neutron star orbit, we obtain a density of $n \approx 3 \times 10^9 \text{ nuclei cm}^{-3}$. For protons, using an attenuation length¹⁴ of 105 g cm^{-2} , we obtain $t_a \approx 7 \times 10^5 \text{ s}$. Using recent data¹⁵ on the spallation of iron on hydrogen we obtain $t_a \approx 4 \times 10^4 \text{ s}$ for iron nuclei from equation (6). These attenuation times are indicated in Fig. 2a by arrows, for comparison with those calculated for photon interactions. If the matter density is as high as we have assumed, the attenuation time at $\sim 3 \times 10^{16}$ eV for interactions with matter is of the same order of magnitude as for photon interactions in Vela X-1. Matter in the stellar wind may provide target material for the nuclear interactions responsible for the γ rays.

The nature of the stellar object in the Cyg X-3 system is not well known, but is probably a helium star¹⁶, as originally proposed by van den Heuvel and de Loore¹⁷. The likely masses¹⁶ of the compact object (and mass of helium star) are in the range $0.1 M_\odot$ ($2.9 M_\odot$) to $10 M_\odot$ ($1.0 M_\odot$). Figure 2b shows the attenuation time of protons in the vicinity of the compact object of Cyg X-3 calculated for these two cases. The results show that for protons to be accelerated to $\sim 3 \times 10^{16}$ eV (γ rays have been observed from Cyg X-3 up to $\sim 10^{16}$ eV), the acceleration time scale must be less than 10^4 s (case A) or 3×10^5 s (case B).

A high IR luminosity¹⁸ has been observed for Cyg X-3 and may be due to material surrounding the object, perhaps a 'cocoon'¹⁹, heated by UV emission to $\sim 10^4$ K, typical of planetary nebulae. The IR radiation density would then be no greater than that of black-body radiation at $\sim 10^4$ K. Using Fig. 1, the attenuation time of protons at 3×10^{16} eV due to such a component is greater than 3×10^5 s. The possible presence of this additional component of the radiation does not then alter the above conclusion.

It should be pointed out that the calculations of the attenuation time presented here were made assuming an isotropic photon distribution. The attenuation time would be somewhat larger than that calculated if the particles were being accelerated in a direction away from the stellar object, avoiding head-on collisions with photons. Also, acceleration could take place more readily in a region behind the compact object partly shadowed from stellar radiation.

In conclusion, by calculating the time scale for photonuclear interactions in Vela X-1 and Cyg X-3 we have been able to obtain upper limits to the acceleration time of protons in these objects at $\sim 3 \times 10^{16}$ eV. These may be used to put constraints on models for the particle acceleration mechanism operating in these objects. The cutoff in the observed γ -ray energy spectrum of Cyg X-3 may be due to a cutoff in the particle spectrum resulting from photonuclear interactions. Alternatively, the

cutoff may be intrinsic, due to a failure of the acceleration mechanism at high energies, or may result from attenuation of the high-energy γ rays by magnetic pair production processes²⁰. If particles accelerated in binary X-ray sources make an important contribution to the observed cosmic radiation, the steepening in the cosmic-ray spectrum and non-rigidity-dependent composition changes²¹ at 10^{15} – 10^{16} eV may be due in part to photonuclear interactions in such objects.

I am in receipt of a Queen Elizabeth II Fellowship which is gratefully acknowledged. I thank Professor J. R. Prescott and Drs R. W. Clay and A. G. Gregory for reading the manuscript.

Received 1 February; accepted 15 May 1984.

- Samorski, M. & Stamm, W. *Astrophys. J. Lett.* **268**, L17–L21 (1983).
- Lloyd-Evans, J. *et al. Nature* **305**, 784–787 (1983).
- Protheroe, R. J., Clay, R. W. & Gerhardt, P. R. *Astrophys. J. Lett.* **280**, L47–L50 (1984).
- Eichler, D. & Vestrand, W. T. *Nature* **307**, 613–614 (1984).
- Wdowczyk, J. & Wolfendale, A. W. *Nature* **305**, 609–610 (1983).
- Ormes, J. F. & Protheroe, R. J. *Astrophys. J.* **272**, 756–764 (1983).
- Armstrong, T. A. *et al. Phys. Rev. D* **5**, 1640–1652 (1972).
- Stecker, F. W. *Phys. Rev. Lett.* **21**, 1016–1018 (1968).
- Blumenthal, G. R. *Phys. Rev. D* **1**, 1596–1602 (1970).
- Puget, J. L., Stecker, F. W. & Bredekamp, J. H. *Astrophys. J.* **205**, 638–654 (1976).
- Bradt, H. V. D. & McClintock, J. E. *A. Rev. Astr. Astrophys.* **21**, 13–66 (1983).
- Rappaport, S. & Joss, P. in *X-ray Astronomy with the Einstein Satellite* (ed. Giacconi, R.) 123 (Reidel, Dordrecht 1981).
- Dupree, A. K. *et al. Astrophys. J.* **238**, 969–981 (1980).
- Protheroe, R. J. *Astrophys. J.* **251**, 387–392 (1981).
- Webber, W. R. & Brautigam, D. A. *Astrophys. J.* **260**, 894–908 (1982).
- Ghosh, P., Elsner, R. F., Weisskopf, M. C. & Sutherland, P. G. *Astrophys. J.* **251**, 230–245 (1981).
- van den Heuvel, E. P. J. & de Loore, C. *Astr. Astrophys.* **25**, 387–395 (1973).
- Mason, K. O. *et al. Astrophys. J.* **207**, 78–87 (1976).
- Vestrand, W. T. & Eichler, D. *Astrophys. J.* **261**, 251–258 (1982).
- Stephens, S. A. & Verma, R. P. *Nature* **308**, 808–830 (1984).
- Protheroe, R. J. *J. Phys. G* **10**, L99–L106 (1984).
- Paczynski, B. *Acta astr.* **21**, 1–14 (1971).

Water-vapour maser emission from galactic nuclei

M. J. Claussen, G. M. Heiligman & K. Y. Lo

Owens Valley Radio Observatory, California Institute of Technology, Pasadena, California 91125, USA

We report here the progress of a survey of galactic nuclei for water-vapour maser emission at 22.235 GHz. We observed 29 late-type galaxies in November 1982, January 1983, and September 1983 using the 40-m radio telescope of the Owens Valley Radio Observatory (OVRO) equipped with a travelling-wave maser receiver¹. We have detected maser emission from four nuclei: NGC4258 (M106), NGC1068 (M77), NGC3034 (M82), and NGC6946. The masers in NGC4258 and NGC1068 are extremely luminous; NGC1068 has the most luminous water-vapour maser yet reported—350 $L_\odot$ assuming isotropic emission. We suggest that the extremely bright nuclear masers indicate an ongoing burst of star formation, and therefore provide a useful probe of the physical conditions of the starburst phenomenon.

The beam size of the telescope at OVRO at 22 GHz is about 100 arc s, which covers a few kiloparsecs at the distances of the galaxies observed. The typical zenith system temperature was 50 K at 22.2 GHz. The spectrometer used was an acousto-optical spectrometer² with a total bandwidth of 100 MHz and resolution ~ 200 kHz, which resulted in a total velocity (v) coverage of $1,300 \text{ km s}^{-1}$ and a velocity resolution of 2.6 km s^{-1} . For each galaxy the telescope was pointed at the optical nucleus. A description of the observational techniques and the complete results of the survey will be given elsewhere.

Spectra of the maser emission from the four galaxies detected are shown in Figs 1 and 2. If the emission is isotropic, the total luminosities in the line are: 350 $L_\odot$ from NGC1068, 120 $L_\odot$ from NGC4258, 3 $L_\odot$ from M82, and $\sim 1 L_\odot$ from NGC6946, with the assumed distances of 16.5, 6.9, 3.5 and 4.5 Mpc, respectively. The observed luminosity of NGC1068 makes this maser the most luminous yet discovered and requires a maser photon rate of $\sim 4 \times 10^{51} \text{ s}^{-1}$. The luminosities of NGC1068 and NGC4258

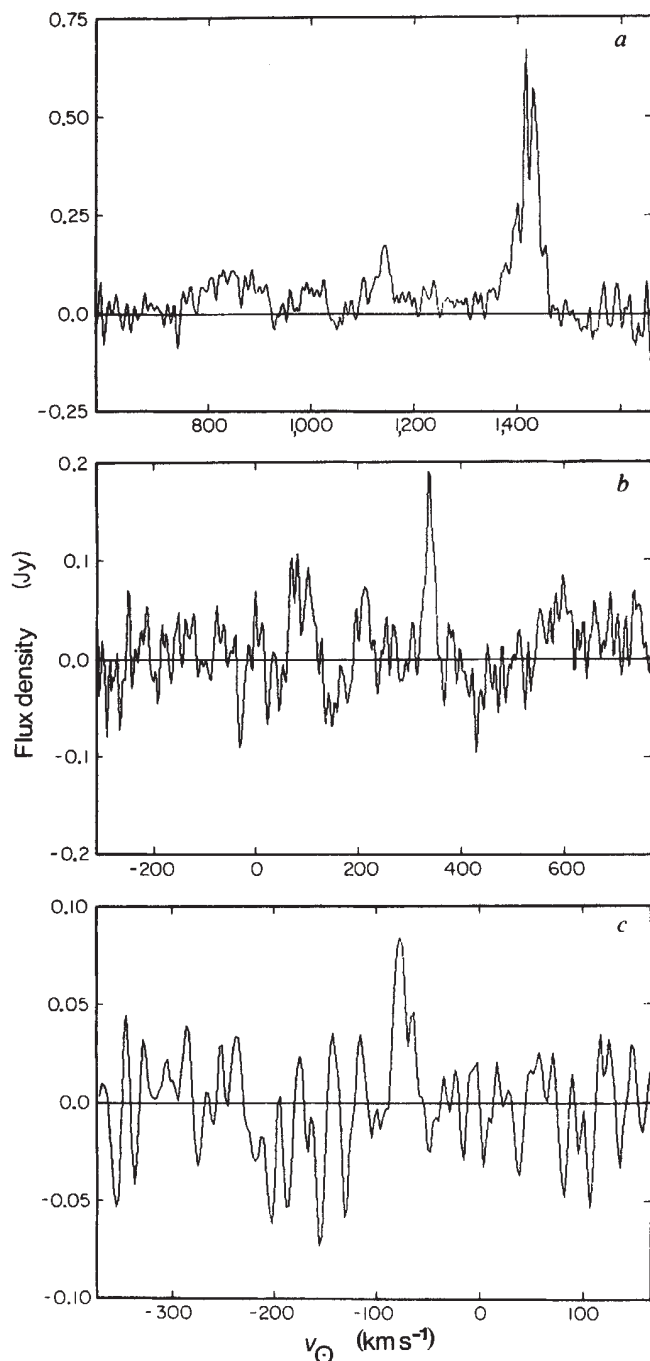


Fig. 1 Spectra of the water-vapour maser emission towards the nuclei of the galaxies NGC1068 (*a*), NGC3034 (*b*), and NGC6946 (*c*) taken with the 40-m OVRO telescope and the acousto-optical spectrometer. The spectral resolution is $\sim 2.6 \text{ km s}^{-1}$ in *a* and *b*, but 5.2 km s^{-1} in *c*.

are larger than or comparable with that of NGC4945 ($165 L_{\odot}$, ref. 3) and the Circinus galaxy ($37 L_{\odot}$, ref. 4). All other confirmed extragalactic water-vapour masers are $\sim 1 L_{\odot}$ or less^{5,6}. For comparison, the total H_2O luminosity from a typical star-forming region in our Galaxy is $\sim 10^{-3} L_{\odot}$ (ref. 7). The strongest galactic source known, W49N, sometimes reaches an isotropic luminosity of $1 L_{\odot}$.

Figure 2 shows the spectrum of the water-vapour maser in NGC4258 taken at epochs two months apart. The feature at $v_0 \sim 460 \text{ km s}^{-1}$ varied by a factor of 2 in that time. We have shown (work in preparation) that this feature resolves into a smoothly rounded peak with $\Delta v = 6.4 \text{ km s}^{-1}$. This result and the variability imply that the emission comes from a single maser 'spot' or group of 'spots'⁸. The isotropic luminosity from this

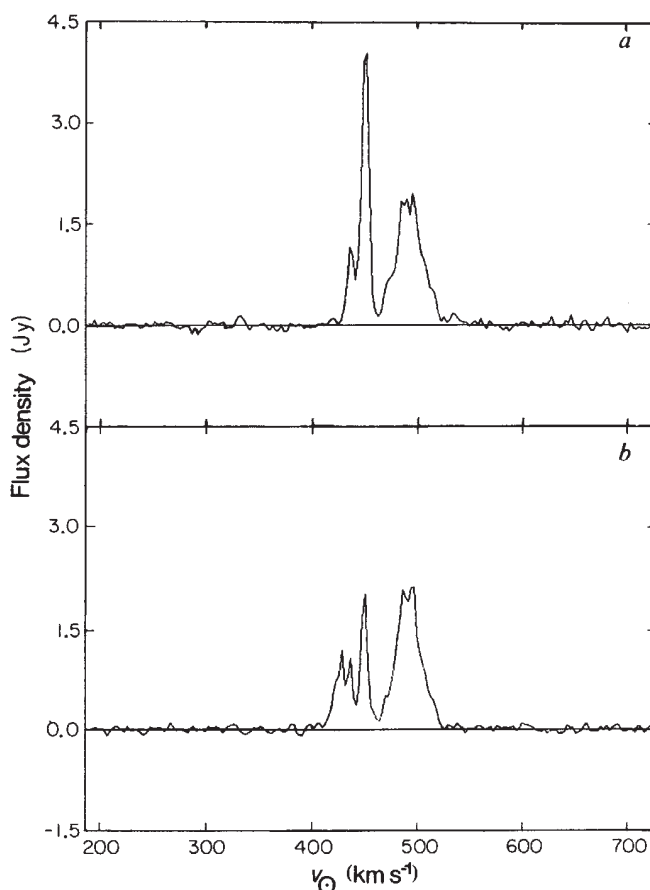


Fig. 2 Spectra of the water-vapour maser emission towards the nuclei of NGC4258 on *a*, November 1982; *b*, January 1983. The spectral resolution is $\sim 2.6 \text{ km s}^{-1}$.

feature alone is $40 L_{\odot}$ and an upper limit to the size based on light-travel time arguments is 10^{17} cm .

The four highly luminous extragalactic water masers (NGC1068, NGC4945, NGC4258, and the Circinus galaxy) are physically different from water masers in our own Galaxy: besides the high total luminosity, the luminosity of each spectral feature detected in these galaxies is greater than $1 L_{\odot}$, and the ensemble of features cover a range of at least 100 km s^{-1} . The spatial extent of the features is not known, but all the very luminous water-vapour maser emission from these four galaxies comes from, at most, a few tens of arcseconds in the nucleus of each galaxy. Thus, there are, within a few kiloparsecs in the nucleus of each of the galaxies detected, at least several water-vapour masers each with luminosity $\geq 1 L_{\odot}$. The maser emission from NGC1068 covers a velocity range $\geq 700 \text{ km s}^{-1}$. The velocity range, which is roughly centred on the systemic velocity ($v_0 = 1,130 \text{ km s}^{-1}$) is greater than that of either the H I emission⁹ ($\sim 500 \text{ km s}^{-1}$, full-width at zero intensity, FWZI) or the CO emission¹⁰ ($\sim 450 \text{ km s}^{-1}$, FWZI) from this galaxy. High-velocity maser emission in galactic sources is known¹¹, but the high-velocity components are always much weaker than low-velocity features. The maser emission from NGC1068 provides a contrast to this as the strongest emission arises near the extreme redshifted velocities. The line profile resembles that of galactic OH masers in expanding circumstellar shells. The H_2O maser emission in NGC1068 may originate in an outflow from a central source.

The discovery of such highly luminous water-vapour masers only exacerbates the problem of explaining the physics of interstellar masers. Several possible explanations for such masers have been discussed¹²⁻¹⁵, but there has been no consensus about the mechanism for producing even the luminous galactic masers such as W49N or the recent H_2O maser flare in Orion¹⁶. It seems, at least for the strong sources, that the masers must be saturated.

Strelnitskij¹⁵ and Downes¹⁴ argue that radiative pumping schemes fail to provide enough pump power. Strelnitskij¹⁵ suggests that the only possible mechanism for producing enough pump power is a collisional pump with two kinds of particles at different kinetic temperatures. This requires H₂ densities fairly close to the limit for quenching the maser by thermalization¹⁴. Deguchi's¹³ suggestion of a collisional pump with cold dust acting as a sink of far IR water-vapour radiation appears to be a more feasible mechanism, but the size scale required for the very luminous masers in his model is rather large compared with the sizes deduced from the VLBI observations of galactic sources. Downes¹⁴ suggestion that the luminosity of a single strong spectral feature comes from many spatially separated spots may be relevant in this case. The sizes of the extragalactic masers are unknown; preliminary observations with the Very Large Array (VLA) suggest that all the emission in NGC4258 (120 L_☉) is confined to a region 70 m arc s in size, corresponding to ~2 pc at the distance of this galaxy. All models of the very luminous masers have three things in common: (1) very high densities ($n_{\text{H}_2} \geq 10^9 \text{ cm}^{-3}$); (2) a heat source to provide pump energy for the maser; and (3) an energy sink to complete the pump cycle and provide the inversion. The heat source is usually but not always postulated to be a strong IR source. The temperature of the heat source in all the models is ~1,000 K, and the temperature difference between the heat source and energy sink is ~200 K.

In general, then, it seems that a neighbouring strong source of heating and very high densities are necessary to produce H₂O maser emission. In the Galaxy, such conditions are found in regions of the interstellar medium where massive star formation occurs. The heat sources for most galactic masers are thought to be luminous IR sources that are typically associated with such regions. The lifetime of the water-vapour maser phase of star formation is estimated to be ~10⁵ yr (ref. 17) based on observations of galactic star-formation regions. For the four galaxies with very luminous masers, the nuclear IR luminosities are $\geq 10^{10} L_{\odot}$ except for NGC4258 which has a nuclear luminosity of $6 \times 10^8 L_{\odot}$ (ref. 18). Each galaxy also shows some evidence for nuclear activity: NGC1068 is the prototypical Type 2 Seyfert galaxy and NGC4258 is classified as having a spectrum intermediate between Seyfert galaxies and Liners¹⁹. NGC4258 has anomalous 'arms' detected in H α (ref. 20) and the radio continuum²¹, which may be indicative of some past nuclear activity^{21,22}. Recent observations of the Circinus Galaxy and NGC4945 also show evidence for nuclear activity²³. We find that high bolometric luminosity and indications of nuclear activity are necessary, but not sufficient conditions, for the presence of highly luminous water masers. M82 is an example of a galaxy with high IR luminosity and indicators of nuclear activity but without a highly luminous water-vapour maser. Thus, the very luminous water-vapour masers are likely to be short-lived phenomena associated with the activity in galactic nuclei.

As the high luminosity masers require a large collection of sources with high density in the proximity of some source of luminous heating, they may imply the concurrent existence of many star-forming regions. Because recent bursts of star formation are frequently invoked to account for the high IR luminosities and activity in galactic nuclei, we suggest that the highly luminous water masers are also a consequence of a high rate of star formation. Furthermore, the very short maser lifetime implies that this phenomenon traces a current burst of star formation. If this suggestion is true, then the very luminous water masers in galactic nuclei provide a diagnostic tool for determining the conditions of the starburst phenomenon. Other, more exotic explanations are possible. For example, Moorwood and Glass²³ have speculated that the formation of a supermassive object in the evolution of the nucleus might account for both Seyfert and H₂O maser activity in NGC4945. Our observations cannot rule out such a conclusion, and indeed, the 460 km s⁻¹ feature in NGC4258 and the masers in NGC1068 may support such a speculation.

We thank the staff of the Owens Valley Radio Observatory, especially Harry Hardebeck, Colin Masson and Steve Scott. This research was supported by grant AST82-10828 to the NSF. The VLA is operated by the National Radio Astronomy Observatory, Associated Universities, Inc. under contract to the NSF.

Received 23 April; accepted 24 May 1984.

- Moore, R. L., Hardebeck, H. E., Moffet, A. T. & Neff, D. *Abstr. at U.S. National Radio Science Meet.*, Boulder (1983).
- Masson, C. R. *Astr. Astrophys.* **114**, 270–274 (1982).
- dos Santos, P. M. & Lepine, J. R. D. *Nature* **278**, 34–45 (1979).
- Gardner, F. F. & Whiteoak, J. B. *Mon. Not. R. astr. Soc.* **201**, 13P–15P (1982).
- Churchwell E. et al. *Astr. Astrophys.* **54**, 969–971 (1977).
- Whiteoak, J. B. et al. *Mon. Not. R. astr. Soc.* **205**, 275–279 (1983).
- Genzel, R. & Downes, D. *Astr. Astrophys. Suppl.* **30**, 145–168 (1977).
- Reid, M. J. & Moran, J. M. A. *Rev. Astr. Astrophys.* **19**, 231–276 (1981).
- Allen, R. J., Darchy, B. F. & Lauque, R. *Astr. Astrophys.* **10**, 198–204 (1971).
- Rickard, L. J., Palmer, P., Morris, M., Turner, B. E. & Zuckerman, B. Z. *Astr. Astrophys. J.* **213**, 673–694 (1977).
- Morris, M. *Astr. Astrophys. J.* **210**, 100–107 (1976).
- Goldreich, P. & Kwan, J. *Astr. Astrophys. J.* **191**, 93–100 (1974).
- Deguchi, S. *Astr. Astrophys. J.* **249**, 145–151 (1981).
- Downes, D. in *Birth and Infancy of Stars* (eds Lucas, R. & Omont, A.) (in the press).
- Strelnitskij, V. S. *Mon. Not. R. astr. Soc.* **207**, 339–354 (1984).
- Abraham, Z., Cohen, N. L., Opher, R., Raffaelli, J. C. & Zisk, S. H. *Astr. Astrophys.* **100**, L10–L13 (1981).
- Genzel, R. & Downes, D. *Astr. Astrophys.* **72**, 234–240 (1979).
- Rieke, G. H. & Lebofsky, M. J. *Astr. Astrophys. J. Lett.* **220**, L37–L41 (1978).
- Heckman, T. M. *Astr. Astrophys.* **87**, 152–164 (1980).
- Courtes, G. & Cruvellier, P. C. *r. hebdom. Séanc. Acad. Sci. Paris* **253**, 218–220 (1961).
- van der Fruit, P. C., Oort, J. H. & Mathewson, D. S. *Astr. Astrophys.* **21**, 169–184 (1972).
- van Albada, G. D. thesis, Univ. Leiden (1978).
- Moorwood, A. F. & Glass, I. S. *Astr. Astrophys.* (submitted).

Orbital inclination and mass of the binary pulsar PSR0655 + 64

A. G. Lyne

University of Manchester, Nuffield Radio Astronomy Laboratories, Jodrell Bank, Macclesfield, Cheshire SK11 9DL, UK

The full determination of the orbits of a binary star system requires measurements of both line-of-sight and transverse velocities. Except for orbits of large angular size, there is usually insufficient angular resolution for transverse velocities to be measured; the orbital parameters then contain an unknown orbital inclination. It is this that has so far limited the determination of the parameters of the binary system containing the pulsar PSR0655 + 64. Recent proper motion measurements have shown that the transverse velocities of pulsars can be determined from observations of the rate of scintillation of pulsar radio intensity due to irregularities in the interstellar medium¹. We report here variations in the rate of scintillation that have been measured as the pulsar PSR0655 + 64 moves around its orbit, allowing a direct determination of the inclination of the orbit, the transverse velocity of the whole binary system through space and also of the linear scale of the interstellar scintillation pattern. The observations also provide an upper limit to the diameter of the companion star and the electron density within the orbit.

PSR0655 + 64 is in an almost exactly circular orbit with an orbital period of 24.7 h. Timing measurements by Damashek et al.² summarized in Table 1 relate only to the line-of-sight component of the velocity, so that the overall scale of the orbit and the stellar masses required to sustain it depend on the unknown inclination, i , of the plane of the orbit to the plane of the sky. Thus, assuming a perfectly circular orbit of radius a_p and period P_b , the true orbital velocity is

$$v_0 = \frac{2\pi a_p}{P_b} = \frac{87.5}{\sin i} \text{ km s}^{-1} \quad (1)$$

If the inclination is small, the orbit will appear almost circular and the speed of the pulsar in the plane of the sky will vary very little and be close to v_0 . However, if the inclination is large the pulsar will appear to oscillate back and forth along a line, and its speed will vary between 0 and v_0 . Clearly the ratio of

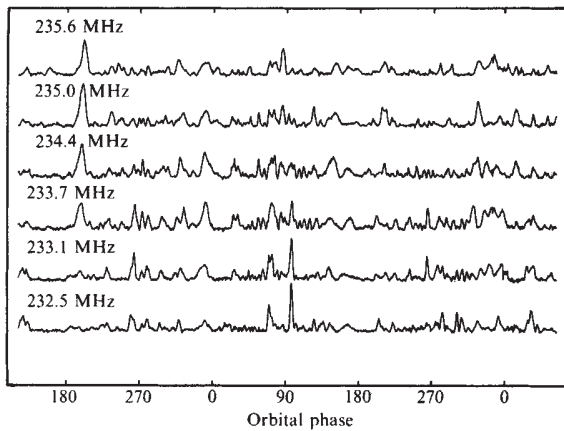


Fig. 1 The scintillation patterns obtained from PSR0655+64 at 234 MHz between 18 November 1983 and 20 November 1983. The six traces are for each of six contiguous frequency bands having a separation and bandwidth of 625 kHz. The orbital phase θ is relative to the position of the ascending node, that is when the pulsar is at one extreme of the major axis of its apparent orbit and moving away from the Earth.

the maximum to minimum speeds is directly related to the orbital inclination.

The pulsar has a dispersion measure (DM) of 8.74 pc cm^{-3} , indicating a distance of about 300 pc. At this distance the orbit would subtend an angle of $<10^{-4}$ arcs at the Earth and be impossible to resolve even with very long baseline radio-interferometry. However, it has been demonstrated recently that the rate of interstellar scintillation of the radiation from a pulsar is directly related to the transverse motion of the line-of-sight to the pulsar relative to the intervening medium¹. We have, therefore, sought to measure the scintillation rate of the pulsar radiation around the orbit.

Preliminary observations in June 1982 at 408 MHz showed that the scintillation rate was rather slow at this frequency and although a variation in rate was apparent in the data it was not possible to determine the variation with any certainty. A lower frequency of observation was thus indicated in order to obtain an increased scintillation rate and hence a better statistical measure of the instantaneous rate. Consequently, observations were made at a frequency of 234 MHz using the 76-m telescope at Jodrell Bank for a total of 90 h between 18 November and 2 December 1983. The total intensity of the pulsar radiation was recorded in each of six contiguous frequency bands of width 625 kHz and integrated over intervals of 2 min. The scintillation patterns obtained for one observation period of nearly 2 days are shown in Fig. 1 as a function of the pulsar orbital phase. The scintillation has a characteristic time scale of about 30 min and a 50% decorrelation frequency of 1.1 MHz. The variation in scintillation rate around the orbit can be seen clearly: the rate is small at phases 0 and 180°, corresponding to the extremities of the orbit where the motion of the pulsar might be expected to be predominantly towards or away from the Earth, and fast near phases 90° and 270° where the transverse motion is expected to be maximal.

The scintillation rate was determined as the mean rate at which the scintillation pattern crosses the time-averaged mean intensity level in a given time interval. The rate as a function of orbital phase is shown in Fig. 2, where the data have been averaged over all 90 h of observation. The scintillation rate varies by more than a factor of 2 around the orbit.

To interpret this variation quantitatively, we consider the transverse motion of the pulsar to consist of not only the orbital velocity v_0 but also a transverse drift velocity vector (v_x, v_y) of the whole binary system, where the major axis of the elliptical apparent pulsar orbit is assumed to be in the positive x -direction. The orbital phase of the pulsar is θ measured relative to the

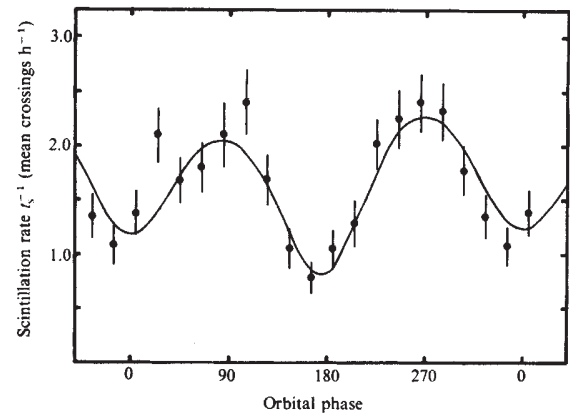


Fig. 2 The variation in scintillation rate as a function of orbital phase θ . The standard deviation of each estimate is indicated. The solid line represents the variation expected for each of the two indistinguishable fits given in Table 2.

ascending node³. Thus the instantaneous transverse velocity of the pulsar in the plane of the sky is given by

$$v_t^2 = (v_x - v_0 \sin \theta)^2 + (v_y + v_0 \cos \theta \cos i)^2$$

If the scale size of the scintillation pattern is a_s , then the observed scintillation rate $1/t_s$ will be given by $1/t_s = v_t/a_s$, leading to

$$\frac{1}{t_s^2} = \frac{v_0^2}{a_s^2} \left[\frac{(v_x^2 + v_y^2)}{v_0^2} + \frac{2 - \sin^2 i}{2} - \frac{2(v_x \sin \theta - v_y \cos \theta \cos i)}{v_0} - \frac{\sin^2 i \cos 2\theta}{2} \right]$$

This displays the two main features of the expected variation of scintillation rate with orbital phase θ . The last term represents a variation at twice the orbital rate and its magnitude is directly related to the inclination of the orbit, i . The middle term oscillates

Table 1 Parameters of the binary pulsar PSR0655+64

Dispersion measure (pc cm^{-3})	DM	8.74
Pulsar rotation period (s)	P	0.196
Period derivative (s s^{-1})	$\dot{P}$	$<10^{-18}$
Orbital period (h)	P_b	24.7
Orbital semi-major axis (light s)	$a_p \sin i$	4.126
Orbital eccentricity	e	$<4 \times 10^{-5}$
Mass function ($M_\odot$)	$\frac{(m_c \sin i)^3}{(m_c + m_p)^2}$	0.0712

Table 2 Parameters of PSR0655+64 determined from scintillation studies

		Solution 1	Solution 2
Orbital inclination (deg)	i	62 ± 5	84 ± 3
Orbital radius (light s)	a_p	4.66 ± 0.19	4.15 ± 0.05
Orbital velocity (km s^{-1})	$v_0 = 87.5/\sin i$	99 ± 4	88 ± 1
Drift velocity* (km s^{-1})	v_d	10 ± 5	46 ± 6
Drift velocity† direction (deg)	ϕ_d	64 ± 25	84 ± 6
Scintillation pattern scale size‡ (km)	a_s	$62,000 \pm 5,000$	$62,000 \pm 5,000$

* $v_d = (v_x^2 + v_y^2)^{1/2}$.

† $\phi_d = \arctan(v_y/v_x)$.

‡ a_s is the distance at which the intensity spatial autocorrelation function falls to 0.5.

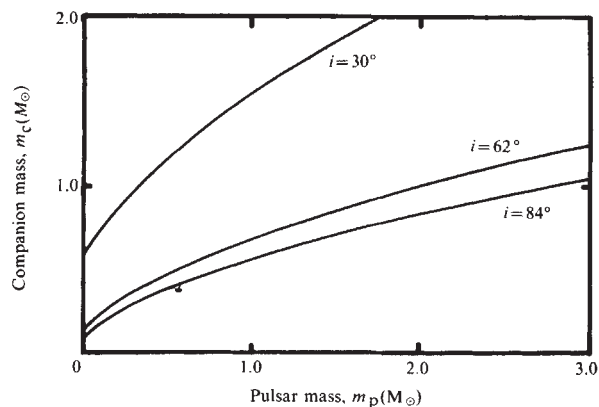


Fig. 3 The relation between the mass of the pulsar, m_p , and the mass of the companion star m_c . The system must lie close to one of the lines $i = 62^\circ$ or $i = 84^\circ$.

at the orbital rate and its magnitude and phase are related to the magnitude and direction of the drift velocity vector. Thus, in principle, it is possible to determine the orbital inclination i and hence the orbital radius a_p and the total orbital velocity v_0 (equation (1)). The drift velocity can also be determined in terms of the now known orbital velocity v_0 . Furthermore, the size and transverse velocity of the orbit are known, and this permits a direct measurement of the scale a_s of the scintillation pattern.

A weighted least-squares fit of t_s to the observed scintillation rate was carried out to obtain the best values of i , v_x/v_0 and v_y/v_0 . There turn out to be two possible solutions which are indistinguishable; the degenerate solution is shown in Fig. 2 and is clearly a good fit to the data. These two solutions are summarized in Table 2.

It is easy to see why there are two solutions. The first solution essentially explains the finite scintillation rate at the extremities of the orbit (that is phases 0° and 180°) as being due to the finite transverse orbital velocity at these phases of the apparent orbit. The second explains the residual velocity in terms of a large apparent eccentricity, the pulsar having low orbital velocity at 0° and 180° , but having a significant drift velocity. It can be shown that there are just two such solutions to each set of observations like this one.

It is possible, in principle, to resolve this ambiguity by further observations at an appropriate time. One can consider the scintillation rate to be determined primarily by the transverse velocity of the line-of-sight between the pulsar and the Earth relative to the intervening interstellar medium. So far we have attributed all the motion to the pulsar, assuming that the Earth is at rest relative to the medium. However, the Earth is moving with a velocity of $\sim 30 \text{ km s}^{-1}$ in its orbit around the Sun and had a velocity of $\sim 27 \text{ km s}^{-1}$ normal to the line-of-sight to the pulsar during the present observations. This vector will contribute to the drift vector found in the two solutions presented in Table 2. Six months after this observation, the velocity vector of the Earth relative to the line-of-sight to the pulsar will be different by $\sim 50 \text{ km s}^{-1}$ in a known direction. Repeating the observations described here at that time will lead to two further solutions for the inclination and drift velocity, one of which will be compatible with one of the given solutions, so resolving the ambiguity. Moreover, the change in drift velocity vector will have the same direction as the known change in the Earth velocity vector, so allowing the position angle of the major axis of the apparent pulsar orbit to be established.

Despite the ambiguity which is present in the interpretation of this current experiment, it is clear that the orbit is highly inclined with $i \geq 60^\circ$ and the total orbital velocity, therefore, $< 100 \text{ km s}^{-1}$.

The binary period and projected semi-major axis yield the mass function $f(m_p, m_c)$ which provides a necessary relationship between the masses of the pulsar m_p and its companion m_c

required to maintain a stable orbit. Thus

$$f(m_p, m_c) = \frac{m_c^3 \sin^3 i}{(m_p + m_c)^2} = \frac{4\pi^2}{G} \frac{(a_p \sin i)^3}{P_b^2} = 0.0712 M_\odot$$

where $M_\odot$ is the solar mass. Since we know that i is either 62° or 84° , then this equation is now constrained and we have

$$\frac{m_c^3}{(m_p + m_c)^2} = 0.103 M_\odot \text{ or } 0.072 M_\odot$$

These relationships between m_p and m_c are represented graphically in Fig. 3 and indicate that for a typical neutron star mass of $1.4 M_\odot$, the companion must have a mass of $< 0.8 M_\odot$.

As there is no sign of any occultation of the pulsar by the companion near to $\theta = 90^\circ$, we can say that the companion is compact. Furthermore, there is no evidence of any dispersing plasma within the orbit, as the values of $a_p \sin i$ at 408 and 234 MHz are the same to within about $50 \mu\text{s}$. This implies a dispersion measure of $\leq 10^{-5} \text{ pc cm}^{-3}$ and a corresponding mean electron density of less than 125 cm^{-3} within the binary orbit. This is rather less than that expected from any main sequence star and it seems that the companion must, therefore, be either another neutron star or a white dwarf.

The evolution of the system has resulted in extremely circular orbits (eccentricity, $e < 4 \times 10^{-5}$). At the time of the primary collapsing to a neutron star, presumably in a supernova event, the binary system was very fortunate to escape disruption either through asymmetry of the core collapse and explosion or because of the sudden mass-loss from the binary system. In either case, it is extremely unlikely that the resulting bound orbit should be highly circular. Some form of circularization of the orbit must subsequently have occurred and could have been the result of either accretion from, or tidal interaction with, the companion star, although neither would have been possible if the companion were as compact as a neutron star or white dwarf. We have to conclude therefore that immediately following the formation of the neutron star the companion was not compact, that subsequently orbital circularization occurred and the companion evolved into the compact object that now exists. This evolutionary sequence must all have occurred within the lifetime of the pulsar, which for pulsars generally is believed to be $\leq 10^7 \text{ yr}$.

Received 16 April; accepted 24 May 1984.

1. Lyne A. G. & Smith, F. G. *Nature* **298**, 825–827 (1982).
2. Damashek, M., Backus, P. R., Taylor, J. H. & Burkhardt, R. K. *Astrophys. J. Lett.* **253** L57–L60 (1982).
3. Smart, W. M. *Spherical Astronomy* Ch. 14 (Cambridge University Press, 1962).

Early Jurassic pillow lavas and palynomorphs in the Karoo of eastern Botswana

D. T. Aldiss,* J. M. Benson† & C. C. Rundle‡

* Geological Survey, Private Bag 14, Lobatse, Botswana

† SOEKOR, PO Box 3087, Johannesburg, South Africa

‡ Isotope Geology Unit, British Geological Survey, 64–78 Gray's Inn Road, London WC1X 8NG, UK

Volcanic rocks in conformable contact with fossiliferous sedimentary strata apparently present excellent possibilities for the calibration of the stratigraphical record by radiometric age determination. Unfortunately, many continental sedimentary sequences are poor in zone fossils. For example, correlation of the desert sediments in the upper Karoo Sequence of southern Africa relies on broad lithostratigraphical divisions¹ although vertebrate fossils can also be used² in some areas. However, precise dating of the onset of Karoo vulcanicity is crucial to the description

of Gondwanaland. Here we note a highly exceptional area in eastern Botswana where the top of the largely aeolian 'Cave Sandstone' and the base of the overlying 'Stormberg Lavas' can be dated by the combined use of palaeontological and radiometric methods. Fluvio-lacustrine sediments within the lowest Stormberg Lavas are conformably overlain by basalts (in places, pillow lavas) with a probable K-Ar minimum age of ~181 Myr. Early Jurassic palynomorphs (*Classopollis intrareticulatus*) suggest that the maximum age of these sediments is in the Upper Sinemurian, between ~195 and 200 Myr.

The Cave Sandstone, at the top of the sedimentary sequence in the Tuli Basin (Fig. 1), is a largely aeolian deposit although small-scale cross-bedding and mud-pellet horizons indicate that at least part of this unit was waterlain (see ref. 3). Although a *Glossopteris* flora and sparse Upper Triassic reptilian remains occur in the older Karoo sediments in Zimbabwe, no fossils have been found in the Cave Sandstone anywhere in the Tuli Basin⁴⁻⁷. The Stormberg Lavas in Botswana are a thick pile of subaerial basaltic lavas with little pyroclastic material. In places towards the southern margin of the Tuli Basin, the lavas were deposited on an irregular surface of sandstone. In some areas this surface has been considered to be erosional and, therefore, to represent an unknown time-gap⁴⁻⁸. Elsewhere, the lavas overwhelmed sand dunes with only slight disconformity⁹.

However, there are widespread sedimentary lenses inter-layered with the lowest of the Stormberg Lavas^{4,5,10}. In the Tuli Basin, these range from a few centimetres thick⁴, to more than 40 m thick with an exposed strike length of 27.5 km (ref. 7). The lower of the two main lenses which occur in the Madikolola River, about 7 km south-east of Bobonong (Fig. 1), is composed of locally argillaceous or carbonaceous quartz sandstone up to 3 m thick which is overlain by a thin discontinuous limestone. The basalts beneath this sandstone layer wedge out to the east and the layer merges with the top of the Cave Sandstone, implying that the latter is partly coeval with the oldest lavas. The upper lens consists of limestone with thin sandstone and mudstone lamellae. It, too, is locally carbonaceous. The limestone draped the underlying lava and it was distorted by the succeeding lava, in which pillow structure occurs. These two lenses are separated by only ~25 m of lavas. In this essentially conformable sequence, there appears to have been only a small time-gap between the deposition of the top of the Cave Sandstone, the sandstone and limestone lenses and the enclosing basalts.

Although this is the first record of Karoo pillow lava in Botswana, it is known at a few other localities in Zimbabwe¹¹ and South Africa^{10,12}, often associated with sedimentary interbeds. Pillow structure only forms when a lava flow enters water (or, very exceptionally, snow or mud)^{10,13}. The pillow lavas at

Table 1 K-Ar ages for basalts from eastern Botswana

Sample	% K	Vol. radiogenic ⁴⁰ Ar (nl g ⁻¹)	Atmospheric ⁴⁰ Ar (%)	Age ± 2 σ (Myr)
BG65	1.48	10.21	18.7	169 ± 4
BG66	0.769	4.91	30.7	158 ± 4
BG69	0.994	6.62	13.2	164 ± 9
BG71	2.12	15.59	8.6	180 ± 4
BG72	2.07	14.29	9.3	170 ± 6*
BG74	2.13	15.85	26.1	182 ± 6*
BG75	2.10	15.63	12.5	182 ± 5*

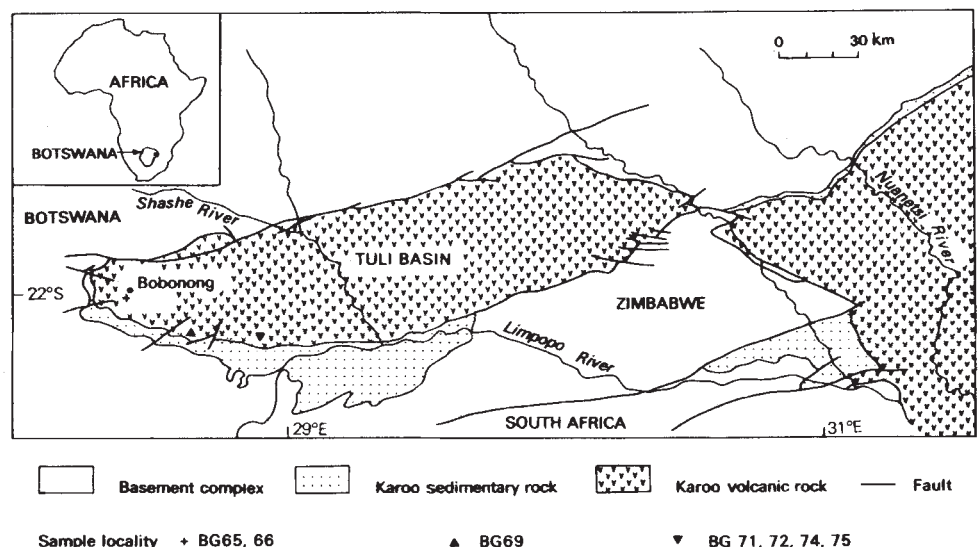
Potassium was determined with a precision of better than ±1% (1 σ) using a digital flame photometer with lithium internal standard. Argon was extracted under vacuum by fusion using radio-frequency induction heating and analysed by the isotope dilution method in a VG Micromass MM 1200 mass spectrometer. Errors on the ages are compounded from all the sources of variation throughout the analysis and also incorporate any replication errors due to sample inhomogeneity. Ages were calculated using the decay and other constants recommended elsewhere²⁷.

* Single argon determination only.

Bobonong are a tiny part of a largely subaerial lava pile overlying continental sediments. It is most likely that they formed where Karoo lava flows entered a lake in which the thin sedimentary lenses were deposited.

The probable palaeogeography of the Bobonong area is suggested by the structure of the Tuli Basin. The Karoo strata in eastern Botswana are preserved in an asymmetric graben, bounded on the northwestern margin by a large fault zone (Fig. 1); this is parallel to pre-Karoo structures in the basement¹⁴ and there is sedimentological evidence for intra-Karoo rifting in Zimbabwe¹⁵ and Botswana^{4,16}. It seems probable, therefore, that during Karoo times the Tuli Basin was a fault-bounded depression and thus the low-lying axis of a drainage basin in which, even during deposition of the largely aeolian Cave Sandstone, some subaqueous sediments were formed. Subsequently, towards the end of Cave Sandstone times, the marginal faults were reactivated. The edges of the basin were uplifted and dissected while deposition continued in the basin axis. When the earliest basalts were extruded they flowed into eroded gullies on the southern margin of the basin and onto an alluvial plain along its axis. After deposition of the lowermost basalts, sedimentation resumed in rivers and lakes, which might well have been lava-dammed. However, the sediment supply seems to have been rapidly choked off by further extrusions, with the onset of limestone deposition. Finally, the lavas filled the depression and sedimentation ceased.

Fig. 1 Location of the Tuli Basin and the study area, after ref. 28. The Karoo sediments include lithological equivalents of the Middle Ecca (oldest), Red Beds and the Cave Sandstone. The analysed samples all come from near the base of the Stormberg Lavas. Stratigraphical nomenclature of the local units is currently being reviewed and formalized by the Geological Survey of Botswana.



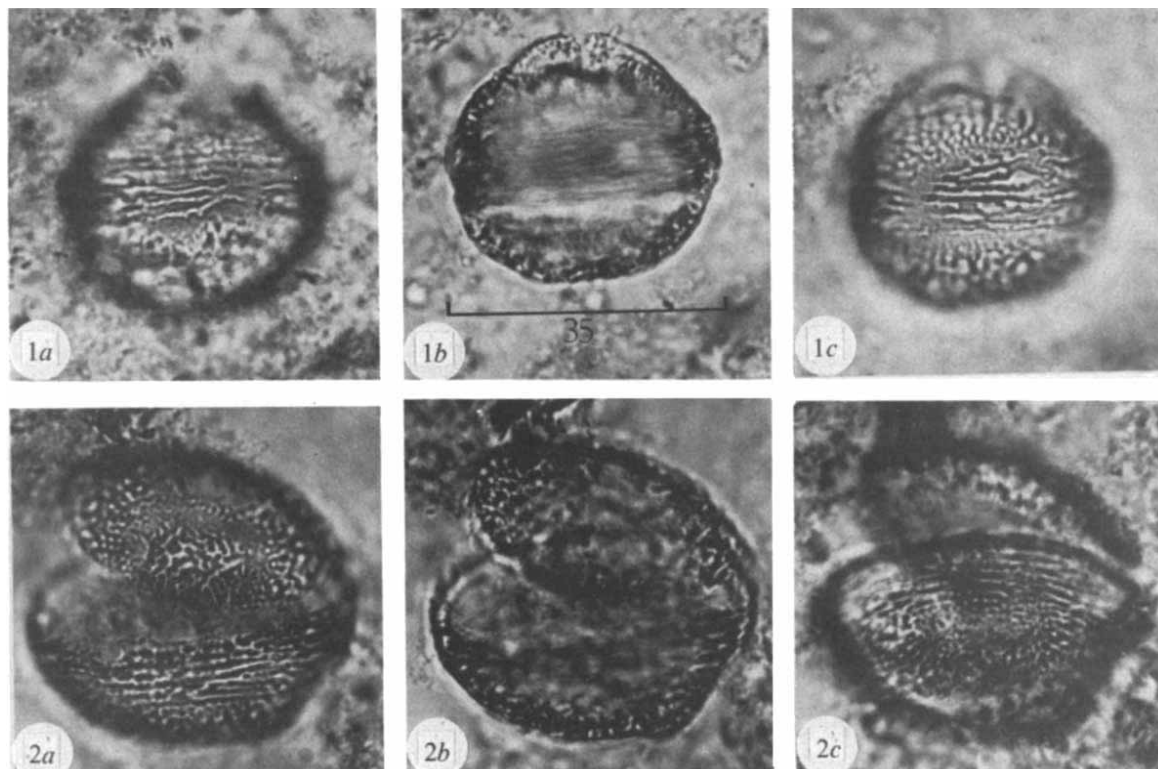


Fig. 2 Two specimens of *Classopollis intrareticulatus* Volkheimer, 1972 at: a, high; b, intermediate; and c, low focus levels in brightfield. $\times 1,000$. Scale bar, 35 μm .

The field evidence and the palaeogeographic model imply that in places the Stormberg Lavas lie conformably on Cave Sandstone, and where they do not the time-gap represented by the disconformity is small. As the time between the deposition of the top of the Cave Sandstone and of the interbedded limestone is also thought to be small (at least relative to the errors in absolute age determinations), suitable fossils in the limestone and radiometric determinations of the associated lavas would effectively 'date' the boundary of the Stormberg Lavas with the Cave Sandstone in eastern Botswana.

A limestone sample from the Madikolola River yielded abundant pollen of the genus *Classopollis* (Pflug, 1953) including *C. intrareticulatus* (Volkheimer, 1972) (Fig. 2), which were produced by plants of the extinct conifer family Cheirolepidiaceae¹⁷⁻¹⁹. This genus has a world-wide distribution with a stratigraphical range from the late Triassic to the Turonian (late Cretaceous)¹⁸. *C. intrareticulatus* has only been found in the range Upper Sinemurian (mid-early Jurassic) to Tithonian (late Jurassic) within sediments of the Neuquén Basin, Argentina^{17,18-22}. This is the first discovery of the genus *Classopollis* in an intracratonic African Karoo basin.

Seven whole-rock samples of Karoo basalt from three localities in eastern Botswana (Fig. 1) were analysed for K-Ar radiometric dating. These are the first such determinations for Botswana. Two of these samples (BG 65 and 66) came from a prominent lava flow which overlies the limestone immediately south of Bobonong. The results in Table 1 suggest a minimum age of 169 ± 4 Myr for this lava flow. However, the other analysed samples from the Tuli Basin have given a range of ages, with three of about 180 Myr. There is a correlation amongst these samples between low potassium contents and low age, suggestive of alteration and probable argon loss (Table 1). In these circumstances it is conventional to assume that the mean of the three oldest results (181 ± 4 Myr) is the best estimate of the minimum age of the samples. This may be compared with K-Ar ages of up to 193 ± 7 Myr for Stormberg lavas from Lesotho²³ and a Rb-Sr age of 190 ± 5 Myr for rhyolites from the Lebombo area of Swaziland²⁴.

Thus, the radiometric data and the fossil evidence can be used together to place close limits on the age of the limestone and hence on the top of the Cave Sandstone. The occurrence of *C. intrareticulatus* in the limestone suggests, given present knowledge, that it is unlikely to be older than Upper Sinemurian. According to the Phanerozoic time scale²⁵, the Upper Sinemurian lies in the range from ~ 195 to 200 Myr. The lava overlying the limestone is probably older than 181 ± 4 Myr (Toarcian). We conclude that the limestone and, therefore, the onset of Karoo vulcanicity are late early Jurassic in age, falling in the range Sinemurian to Toarcian.

The field location of all analysed samples and of known examples of pillow lava referred to here are described in ref. 26.

Received 19 December 1983; accepted 1 June 1984.

1. Miller, J. A. & Fitch, F. J. *Q. J. geol. Soc. Lond.* **120**, 101-116 (1964).
2. Lambert, R. StJ. *Spec. Publ. geol. Soc. Lond.* **5**, 9-31 (1971).
3. South African Committee for Stratigraphy *Stratigraphy of South Africa*, Pt 1 Vol. 8 (1980).
4. Olsen, P. E. & Galton, P. M. *Science* **197**, 983-986 (1977).
5. Mason, R. *Rec. geol. Surv. Bech.* **1961-62**, 25-42 (1965).
6. Green, D. *Bull. geol. Surv. Botswana* **2**, 74 (1966).
7. Aldiss, D. T. *Bull. geol. Surv. Botswana* (submitted).
8. Eriksson, P. G. *Trans. geol. Soc. S. Afr.* **84**, 7-17 (1981).
9. Bond, G. & Falcon, R. M. S. *Bull. Rhodesia geol. Surv.* **70**, 121 (1973).
10. Watkeys, M. K. *Rhodesia geol. Surv. Short Rep.* **45**, (1979).
11. Thompson, A. O. *Rhodesia geol. Surv. Short Rep.* **40**, 79 (1975).
12. Clark, G. C. & Machacha, T. P. *Bull. geol. Surv. Botswana* **19**, 65 (1982).
13. Cox, K. G. *et al. Phil. Trans. R. Soc. A* **257**, 71-218 (1965).
14. Lock, B. E., Paverd, A. L. & Broderick, T. J. *Trans. geol. Soc. S. Afr.* **77**, 117-129 (1974).
15. Bond, G., Wilson, J. F. & Raath, M. A. *Nature* **227**, 1339 (1970).
16. McCarthy, M. J. *Proc. 2nd Gondwana Symp.*, S. Afr. 433-440 (1970).
17. MacDonald, G. A. *Volcanoes* (Prentice-Hall, New Jersey, 1972).
18. Cox, K. G., Bell, J. D. & Pankhurst, R. J. *The Interpretation of Igneous Rocks* (George Allen and Unwin, London, 1979).
19. Aldiss, D. T. *Bull. geol. Surv. Botswana* **25**, (in the press).
20. Reeves, C. V. *Nature* **273**, 222-223 (1978).
21. Volkheimer, W. & Quattrocchio, M. *Ameghiniana* **12**, 193-241 (1975).
22. Volkheimer, W. *Munster. Forsch. Geol. Paläont.* **20/21**, 297-321 (1971).
23. Fitch, F. J. & Miller, J. A. *Bull. Volcan.* **35**, 64-84 (1971).
24. Cleverly, R. W. *Trans. geol. Soc. S. Afr.* **82**, 343-348 (1979).
25. Odin, G. S. (ed.) *Numerical Dating in Stratigraphy* (Wiley, New York, 1982).
26. Aldiss, D. T. *Int. Rep. geol. Surv. Botswana DTA/10/84* (1984).
27. Steiger, R. H. & Jäger, E. *Earth planet. Sci. Lett.* **36**, 341-362 (1977).
28. Vail, J. R., Hornung, G. & Cox, K. G. *Bull. Volcan.* **33**, 398-418 (1969).

Geomagnetic intensity in Egypt and western Asia during the second millennium BC

M. J. Aitken, A. L. Allsop, G. D. Bussell
& M. B. Winter

Research Laboratory for Archaeology and the History of Art,
6 Keble Road, Oxford OX1 3QJ, UK

We report here detailed evidence that during the period 1600–1100 BC the geomagnetic intensity in Egypt and western Asia increased monotonically by a factor of 1.6 and propose that, within this region and this period, such intensity can be used as a dating tool. The results, which form the reference data for age evaluation, are from well-dated archaeological samples of baked clay from Cyprus, Egypt, Israel and Mesopotamia using a version of the Thellier technique¹ as modified for use in a cryogenic (SQUID) magnetometer². There is no evidence for the more rapid fluctuations that have been reported for Egypt³, Greece⁴ and France⁵ during this and other periods. Earlier than 1700 BC the intensity remains close to the present-day level, although there is some indication of a small fluctuation.

Many of the samples were Mesopotamian royal bricks⁶ and Egyptian funerary cones⁷ carrying inscriptions. These have been widely studied and most of the inscriptions are firmly related either to regnal or dynastic lists. The absolute chronology of these lists is based on recorded astronomical events, the dates of which have been calculated in calendar years with relatively small margins of error. For Mesopotamia, we have followed the revised Cambridge Ancient History in using the 'middle' chronology⁸. The remainder of the samples are pottery sherds from firmly-dated levels on archaeological sites, with stylistic confirmation of attribution where possible. The dating is based on the absolute chronology referred to above by means of archaeological linkages; there is no involvement of radiocarbon. The samples on which the final reference data are based are listed in Table 1.

Sampling was carried out using a diamond-impregnated corer in a small hand-held drill, and several small cylindrical cores, 3 mm × 3 mm, were thus obtained from each piece: the exact measurement procedure has been described previously⁹. To be acceptable, a sample must fulfill strict, objective reliability criteria such as discussed elsewhere¹⁰. Also, to avoid samples in which the ancient value might have been distorted by an internal field, an upper limit of $10^{-4} F_A / \cos^2 \gamma \text{ Am}^2 \text{ kg}^{-1}$ is set for the specific magnetization, where F_A is the ancient field intensity (in μT) and γ is the angle between the ancient moment and the perpendicular to the plane of the pottery or brick. If the sample does not have a well-defined plane, the $\cos \gamma$ term is omitted, making the limitation more restrictive. The final criterion is that the ancient field obtained from at least two cores must not differ by more than 5%. Evidence that these criteria are effective is provided by the agreement between contemporary samples having different magnetic characteristics and origins, such as the group which centres on 1300 BC (lines 15–21 of Table 1). The results are corrected for anisotropy⁹ and for the influence of cooling rate¹¹; whereas during measurement the laboratory thermoremanent magnetization (TRM) is imposed during rapid cooling, of the order of $100^\circ\text{C min}^{-1}$, the cooling in antiquity probably took place over a few hours or a few days. Fortunately, the dependence on rate is weak; typically the apparent field obtained directly from the measurements needs to be reduced by 5.5% to get the true field if the cooling is over 4 h, and 8% if over 2 days. For individual samples the corrections may be as low as zero and as high as 9 and 12% respectively.

Samples that passed all the criteria were subdivided into grades 1 and 2 according to their closeness to the limits, the latter being nearer the borderline. Samples for which the cores differ by between 5 and 10% and do not quite fulfill the other criteria are categorized as grade 3, all others being rejected. The grade 3 results showed more scatter but indicated the same trend. The grade 1 and 2 results are plotted in Fig. 1 as the ratio between the ancient intensity and the intensity at the site due to an axial, centred, geomagnetic dipole of present-day strength ($8 \times 10^{22} \text{ Am}^2$), thus making allowance for latitude dependence of the dipole component of the field.¹² As the scatter of the

Table 1 Geomagnetic intensity reference samples and results for the second millennium BC

Sample	Location	Presumed date BC	Intensity ratio	F_A (μT)
1 Royal brick (Ur-Ninurta)	Babylonia	1923–1896	0.91	38.5
2 Royal brick (Erishum I)	Babylonia	~1900	0.85	35.8
3 Royal brick (Sin Kashid)	Babylonia	~1860–1830	0.95	40.2
4 Royal brick (Sin Iddinam)	Babylonia	~1849–1843	0.90	38.1
5 Royal brick (Silli Adad)	Babylonia	1835	0.92	38.9
6 Four Royal bricks (Hammurabi)	Babylonia	1792–1750	1.03, 1.03, 0.99, 0.9	43.6, 43.7, 42.0, 40.1
7 Three sherds (Palace floor)	Tell al Rimah, N. Iraq	1780–1750	0.75, 1.00, 0.86	33.4, 43.9, 37.8
8 Three sherds (Destruction level)	Tell al Rimah, N. Iraq	1760–1750	0.99, 1.04, 0.90	43.7, 36.1
9 Two sherds (level c/2)	Tell el-Dab'a, N. Egypt	1750–1700	0.89, 0.87	37.0, 36.1
10 Two sherds (level E/1)	Tell el-Dab'a, N. Egypt	1630–1590	0.98, 1.04	40.6, 43.0
11 Two sherds (Destruction level)	Apheq, Israel	1550–1500	1.06, 1.15	44.9, 48.8
12 Funerary cone (Benia)	Thebes, Egypt	1550–1401	1.29	49.9
13 Two sherds (Tuthmosis)	Karnak, Egypt	1504–1492	1.26, 1.17	48.9, 45.3
14 Royal brick (Karaindash)	Babylonia	~1400	1.23	51.8
15 Four sherds	Tell el Amarna, Egypt	1353–1335	1.31, 1.29, 1.37, 1.41	52.2, 51.6, 54.8, 56.4
16 Two sherds (Seti I Gurna)	Karnak, Egypt	1306–1290	1.29, 1.38	49.7, 53.6
17 Two sherds (level IV)	Kition, Cyprus	1300–1220	1.36, 1.38	60.0, 60.7
18 Royal brick (Shalamaneser I)	Assyria	1273–1244	1.34	59.9
19 Three Royal bricks (Untas Gal)	Elam	~1250	1.44, 1.53, 1.47	60.3, 64.0, 61.4
20 Sherd (destruction layer)	Apheq, Israel	1260–1240	1.38	58.6
21 Royal brick (Tukulti-Ninurta I)	Assyria	1243–1207	1.36	60.6
22 Two sherds (deposit XIII)	Karnak, Egypt	1250–1150	1.51, 1.53	58.4, 59.1
23 Sherd (level II)	Kition, Cyprus	1150–1075	1.62	71.6
24 Royal brick (Tiglath Pileser I)	Assyria	1114–1076	1.50	66.7
25 Three sherds (Pinedjem I)	Karnak, Egypt	~1100–950	1.52, 1.52, 1.51	58.6, 58.5, 58.1
26 Two sherds (CG I)	Kouklia, Cyprus	~1050–1000	1.53, 1.66	67.5, 73.1

The intensity ratio quoted is F_A/F_D , where F_A is the ancient intensity and F_D is the intensity at the site due to an axial centred geomagnetic dipole of present day strength ($8 \times 10^{22} \text{ Am}^2$). Only those samples are listed which were graded 1 or 2 (on a scale 1–6) on the basis of reliability, grade 1 results being underlined. The Mesopotamian chronology used is that of Brinkman, that is, the so-called 'middle' chronology for Hammurabi; the Egyptian chronology used is that of Baines and Malek¹³.

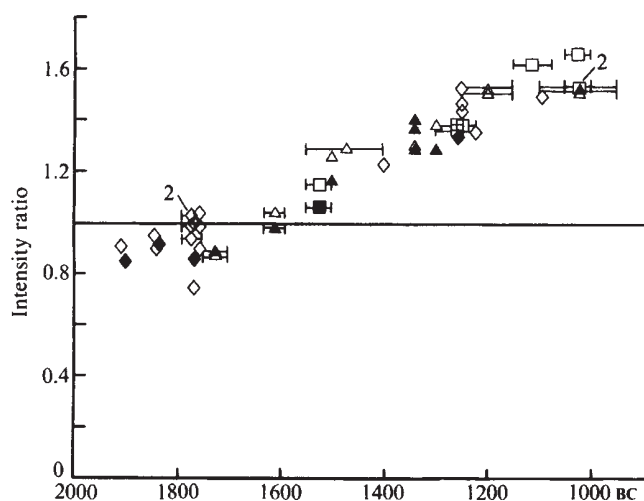


Fig. 1 Geomagnetic field intensity for Egypt and western Asia expressed as the ratio between ancient intensity and the intensity at the site due to an axial, centred, geomagnetic dipole of present-day strength. $\diamond$, Mesopotamia; $\triangle$, Egypt; $\square$, Israel and Cyprus; closed symbols are grade 1 reliability, and open, grade 2. A number adjacent to a data point indicates that it represents more than one sample. Where no age span is indicated the archaeological age is ± 15 yr. Ages used are given in Table 1.

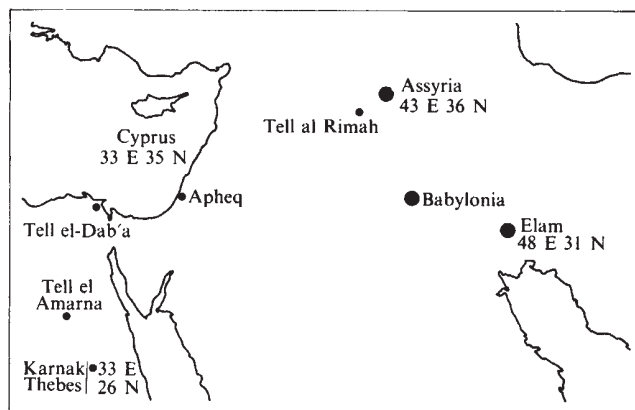


Fig. 2 Region from which the geomagnetic intensity samples originated. The ancient kingdoms of Assyria, Babylonia and Elam are indicated by centrally-placed markers rather than by historical regional boundaries.

points in Fig. 1 is comparable with our estimate of the present overall accuracy ($\pm 5\%$), there is no evidence for geographical dependence of this ratio within the region studied—bounded by Egyptian Thebes (33° E, 26° N) in the south-west, Cyprus (33° E, 35° N) in the north-west, the ancient kingdom of Assyria in northern Iraq (43° E, 36° N) in the north-east, and the ancient kingdom of Elam in Khuzistan (48° E, 31° N) in the south-east (Fig. 2).

We conclude that within this region, and for a group of archaeological objects broadly attributable to 1600–1100 BC on other evidence, the geomagnetic intensity recorded in pottery, bricks and tiles can be useful in giving a more accurate date, approaching ± 50 yr; such an application is now being made. Also, except for centuries in which the ratio is close to unity, the ancient intensity can provide confirmatory evidence in respect of tests for authenticity made by the thermoluminescence method.

We thank Drs P. R. S. Moorey, P. Beck, M. Bietak, V. Hankey, J. Bourriau, H. Jacquet-Gordon, V. Karageorghis, M. Kokhavi, M. Popham, A. J. Spencer and C. B. F. Walker for archaeological collaboration. We also thank the Keeper of Western Asian Antiquities of the British Museum, and the Keeper of Antiquities of the Ashmolean for permission to sample. Financial support

from the Science-based Archaeology Committee of the SERC is acknowledged.

Received 28 March, accepted 23 May 1984.

1. Thellier, E. & Thellier, O. *Ann. Geophys.* **15**, 285–376 (1959).
2. Walton, D. *Archaeometry* **19**, 192–200 (1977).
3. Games, K. P. *Geophys. J.R. astr. Soc.* **63**, 45–56 (1980).
4. Walton, D. *Nature* **277**, 643–644 (1979).
5. Shaw, J. *Geophys. J.R. astr. Soc.* **58**, 107–116 (1979).
6. Walker, C. F. C. *Cuneiform Brick Inscriptions in the British Museum. The Ashmolean Museum. Oxford* (British Museum Publ. No. 71, 1981).
7. Macadam, F. L. *Corpus of Egyptian Funerary Cones* (Oxford University Press, 1957).
8. Brinkman, J. A. in *Ancient Mesopotamia* (by Oppenheim, A. L.) 335–348 (University of Chicago Press, 1977).
9. Aitken, M. J., Alcock, P. A., Bussell, G. D. & Shaw, C. J. *Archaeometry* **23**, 53–64 (1981).
10. Aitken, M. J., Alcock, P. A., Bussell, G. D. & Shaw, C. J. in *Geomagnetism of Baked Clays and Recent Sediments* (eds Creer, K. M., Tucholka, P. & Barton, C. E.) 122–127 (Elsevier, Amsterdam, 1983).
11. Fox, J. M. W. & Aitken, M. J. *Nature* **283**, 462–463 (1980).
12. Barton, C. E., Merrill, R. T. & Barbetti, M. *Phys. Earth planet. Inter.* **20**, 96–110 (1979).
13. Baines, J. & Malek, J. *Atlas of Ancient Egypt* (Phaidon, London, 1980).

Microwave probing of electronic processes in small particle suspensions

John M. Warman & Matthijs P. de Haas

Interuniversity Reactor Institute, Mekelweg 15, 2629 JB Delft, The Netherlands

Michael Grätzel & Pierre P. Infelta

Institut de Chimie Physique, Ecole Polytechnique Fédérale, CH-1015 Lausanne, Switzerland

The electronic properties of small particles of semiconducting materials have attracted considerable attention because they can mediate the photolytic decomposition of water^{1–6}; a property which may prove useful in solar energy storage devices. Information about the initial electron–hole state formed on photolysis and the subsequent conduction and electron transfer processes occurring at the particle–solvent interface has previously been gained indirectly through steady-state and flash-photolysis studies of colloidal solutions that also contain well known electron donor and/or acceptor molecules. A more direct, ‘electrical’ method of probing such systems might be expected to lead to a more detailed understanding of the primary photoionization event and the ensuing bulk and surface electronic processes. We demonstrate here the feasibility of such direct probing of microscopic particles of semiconductor materials (TiO_2 , CdS and Se) suspended in a nonpolar liquid medium.

There are two basic problems associated with electrical measurements on individual, microscopic ($\sim 1 \mu\text{m}$ or less) particles. First, even if a relatively low estimate of $10^{-4} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ is taken for the mobility (μ) of an electronic charge carrier, diffusion from the bulk to the surface of such a particle will occur on a time scale much shorter than a microsecond. Entrapment at the surface, possibly in localized states, can therefore occur very rapidly so that very fast perturbation and detection techniques are needed, if the primary electron–hole pairs are to be observed. The second problem, also due to the small dimensions involved, is that the probing electric field E should only be applied in any given direction for a time, Δt_E , much shorter than the time required for the charge carrier(s) to drift a distance in the field direction which is comparable with the dimensions of the particle. This produces the condition $\Delta t_E \ll d \times 10^{-6} / \mu E$ where d is the dimension of the particle in micrometres. Even for a relatively low field strength of 10^4 V m^{-1} and a not abnormally high mobility of $0.1 \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, Δt_E should be $\ll 1$ ns for microscopic systems. Ultrahigh frequency field modulation is, therefore, a prerequisite for a non-perturbing probe technique.

We have attempted to study the electronic properties of small particles of the semiconducting compounds TiO_2 , CdS and Se

suspended in a liquid-saturated hydrocarbon medium (trans-decalin). The potential problems described above were reduced as much as possible by using a 10-ns (FWHM) pulse of UV light (308 nm) from an excimer laser (Lambda Physik EMG 500) to induce band-gap transitions in the particles and, by measuring any resulting conductivity change as a change in the microwave dielectric loss of the medium with a detection risetime of 5 ns.

The time-resolved microwave conductivity technique was identical to that recently developed for studying photo-induced intramolecular charge separation processes (see ref. 7). For the microwave frequency (9.6 GHz) and power level (100 mW) used, the reciprocal radian frequency of the field ($1/2\pi f$) and maximum field strength in the measurement cavity were 17 ps and $9 \times 10^3 \text{ V m}^{-1}$ respectively. The small perturbation condition given above is, therefore, amply satisfied for particles of micrometre dimensions.

The suspensions were produced simply by shaking the powders and the trans-decalin liquid together by hand. For the first few times the suspension was also sonicated to help disperse any aggregates. The suspensions were not de-aerated. The transmission at 308 nm for freshly shaken samples was 21% (TiO_2), 30% (CdS) and 72% (Se) and remained stable within a few per cent over a time scale of at least several minutes. A degree of aggregation was apparent for TiO_2 . The powders were obtained commercially: TiO_2 (Degussa P25, $55 \text{ m}^2 \text{ g}^{-1}$), CdS (Alpha, $2 \text{ m}^2 \text{ g}^{-1}$) and Se (Fluka purum). No background conductivity was found for CdS and Se suspensions. The TiO_2 suspension did, however, display a small loss, presumably due to extrinsic carriers resulting from oxide deficiency⁸.

Single-shot flash photolysis (10 mJ cm^{-2} per pulse) of all three suspensions resulted in readily measurable changes in the microwave conductivity (dielectric loss) of the medium. This is illustrated by the transient digitizer traces shown in Fig. 1. No conductivity change was observed on flash photolysis of the solvent alone. In addition, the changes are even greater than the largest which have previously been found for the formation of highly dipolar ($\sim 25 \text{ D}$) excited states of molecular charge transfer compounds in the same flash photolysis conditions^{7,9,10}. We conclude, therefore, that the conductivity observed must be due to electron-hole pair formation in the particles of the suspension rather than to photon absorption by spurious molecular impurities. Only monophotonic processes are expected to take place for the photon intensity used. On the other hand, many electron-hole pairs per particle may be formed during the pulse.

A full quantitative analysis of the absolute conductivity values and the rather complex kinetic decay processes is in progress and will be reported elsewhere. We restrict ourselves here to a few comments on the qualitative aspects of the data.

First, the short-time data display an initial rise which is much more rapid than the integrated pulse shape convoluted with the 5-ns time response, as would be expected if the formation of a single, long-lived conducting species were responsible for the transient conductivity change. This effect, and, in addition, a rapid decrease immediately following the pulse, is particularly apparent for the selenium sample. Illumination apparently results initially in a more conducting, but relatively short-lived, state which decays within a time shorter than, or comparable to, the pulse length to yield a much longer-lived conducting state. It seems reasonable to consider that the initial state is that resulting from primary electron-hole formation within the confines of the particle. The short lifetime can then be ascribed to a combination of bulk electron-hole recombination and rapid diffusion to the surface. This would agree with the short lifetime, $< 5 \text{ ns}$, found for fluorescence emission from CdS suspensions^{11,12}.

The persistence of the conductivity transients from tens of nanoseconds to many microseconds is of particular interest as this probably reflects the potential for high charge mobility, even on the particle surface. While this is perhaps to be expected for perfect, uniform two-dimensional surfaces it was by no

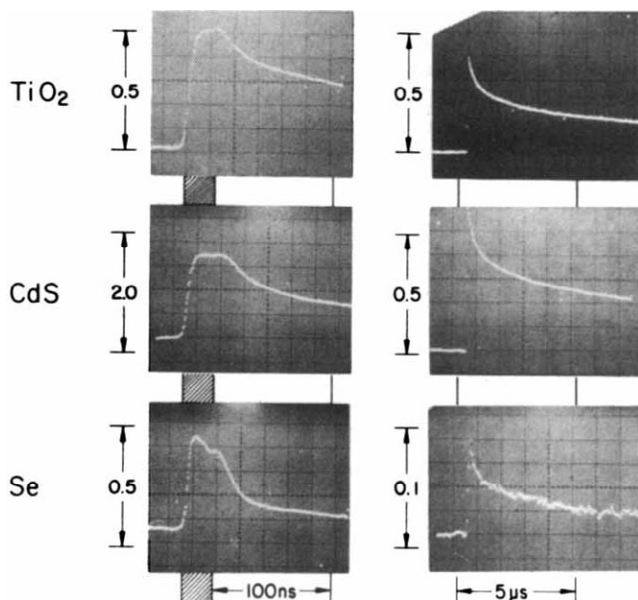


Fig. 1 Transient digitizer traces of the reduction in microwave power reflected by a cell containing particles of TiO_2 , CdS or Se suspended in liquid trans-decalin which results on flash photolysis at 308 nm. The numbers on the left of the traces indicate the sensitivity in terms of the percentage change in reflected power. The shaded region encompasses the 10% intensity points of the laser pulse.

means a forgone conclusion for real, two-dimensional, interfaces. If conduction is, in fact, being observed at the solid-liquid interface, then the present technique can be added to the few which are capable of probing such regions at the molecular level.

That the long-time conductivity transients are due to surface states is most strongly supported by additional results obtained for TiO_2 using the more polar solvent paradioxane. These show an order of magnitude slower decay compared with decalin, although the initial processes ($< 50 \text{ ns}$) are very similar. Such an effect of the surrounding medium on the decay kinetics of the conductivity is difficult to explain if surface states are not involved. On the basis of the available results, however, it cannot be decided whether the effect of solvent is due to a change in the nature of the surface states of individual particles alone or to changes in the static and dynamic interactions between particles.

The decay of the conductivity, even in the long-time domain, is complex and cannot be described by either simple first- or second-order homogeneous three-dimensional kinetics. A logarithmic time dependence similar to that found for charge carriers in amorphous semiconductors gives a better description. This could be due to the sub-three-dimensional movement of the charge carriers and/or to a distribution of partially localized states on the particle surface.

While the technique is limited, at present, to low-loss suspension media, thus excluding polar liquids such as water, it should be a useful tool for gaining a better fundamental insight into the influence of various parameters on the electronics of small particles including, in particular, the intricacies of the semiconductor-liquid interface. The effects of medium, particle size, surface dopants and the presence of dissolved electron and hole transfer agents should be areas particularly worthy of study.

We thank the laser flash photolysis group of Dr C. A. G. O. Varma at the University of Leiden for assistance and use of facilities.

Note added in proof: The surface states of the untreated powder samples used are ill-defined and could be considerably influenced by the presence of adsorbed species, in particular H_2O due to exposure to the atmosphere. With respect to this problem, in experiments carried out after submission of this letter, we have studied the effect of a surfactant (aerosol-OT) entrapped

water layer on the photoconductivity of a TiO_2 suspension. The long-time, 'surface' conductivity signal is indeed found to be considerably influenced (reduced) by the presence of surface water in particular as the layer thickness is increased from 5 to 10 Å. The initial, 'bulk' conductivity signal remains unaffected by water as would be expected.

Received 9 April; accepted 17 May 1984.

1. Inoue, T., Fujiishima, A., Konishi, S. & Honda, K. *Nature* **277**, 637–638 (1979).
2. Duonghong, D., Borgarello, E. & Grätzel, M. *J. Am. chem. Soc.* **103**, 4685–4690 (1981).
3. Darwent, J. R. & Porter, G. *JCS Chem. Commun.* 145–146 (1981).
4. Kalayanasundaram, K., Borgarello, E. & Grätzel, M., *M. Helv. chim. Acta* **64**, 362–367 (1981).
5. Henglein, A. in *Photochemical Conversion and Storage of Solar Energy* Pt A, 115 (ed. Rabani, J.) (Weizmann Science Press, Israel, 1982).
6. Grätzel, M. (ed.) *Energy Resources through Photochemistry and Catalysis* (Academic, New York, 1983).
7. de Haas, M. P. & Warman, J. M. *Chem. Phys.* **73**, 35–53 (1982).
8. Yahia, J. *Phys. Rev.* **130**, 1711–1719 (1963).
9. Warman, J. M., de Haas, M. P., Hummel, A., Varma, C. A. G. O. & van Zeyl, P. H. M. *Chem. Phys. Lett.* **87**, 83–86 (1982).
10. Visser, R. J., Weisenborn, P. C. M., Varma, C. A. G. O., de Haas, M. P. & Warman, J. M. *Chem. Phys. Lett.* **104**, 38–45 (1984).
11. Rossetti, R. & Brus, L. *J. phys. Chem.* **86**, 4470–4472 (1982).
12. Henglein, A. *Ber. Bunsenges. Phys. Chem.* **86**, 301–305 (1982).

Short-term changes in the base neutralizing capacity of an acid Adirondack lake, New York

C. T. Driscoll & G. C. Schafran

Department of Civil Engineering, Syracuse University, Syracuse, New York 13210, USA

Concern and controversy over the effects of acidic deposition on low ionic strength surface waters has led to much discussion on the nature and extent of proton transformations within acid sensitive ecosystems^{1–4}. The source of base neutralizing capacity (BNC) within acid surface waters has been attributed to atmospheric deposition of H_2SO_4 (or SO_2)^{5,6} or HNO_3 (ref. 7), as well as production of soluble organic acids from soils⁸. Unfortunately many of these studies have failed adequately to characterize aluminium, which is often a very significant component of BNC in acidic waters⁹. We have evaluated the nature of short-term changes in the BNC of an acidic clearwater lake. Our results suggest that much of the variation in hydrogen ion and aluminium BNC can be attributed to changes in nitrate concentration, rather than to variations in sulphate, chloride, or organic anion concentrations.

The study site, Dart Lake, is located in the Adirondack Mountain region of New York State, USA (72°52' W, 43°48' N). The watershed drainage area is 107 km², and the lake volume is $1.02 \times 10^7 \text{ m}^3$ with a mean depth of 7.1 m. Dart Lake has a single outlet and our water budget indicates that 95% of the outlet flow ($7.25 \times 10^7 \text{ m}^3 \text{ yr}^{-1}$) enters the lake at the major inlet. Samples were collected for water quality analysis at the inlet, outlet and at seven depths, from a pelagic sampling station, approximately every 2 weeks over an annual cycle (25 October 1981–21 November 1982). Shortly after collection, samples were measured for pH and dissolved inorganic carbon (DIC), amputated for dissolved organic carbon (DOC) analysis, and extracted for monomeric aluminium using the procedure of Barnes¹⁰. Monomeric aluminium was fractionated into labile and non-labile forms using the cation exchange column method of Driscoll^{11,12}. Samples were analysed in the laboratory for all major solutes¹³.

Selected samples, collected from drainage waters in the Dart Lake watershed, were processed and titrated⁹ to characterize the proton dissociation of naturally occurring organic solutes. These data were fitted to a monoprotic proton dissociation model using a modified Gran plot analysis⁹. A statistically significant empirical relationship was observed between DOC concentration and mols of proton dissociation sites per litre ($C_T = 0.025 \text{ DOC} + 8.0$; where C_T is the proton dissociation sites on

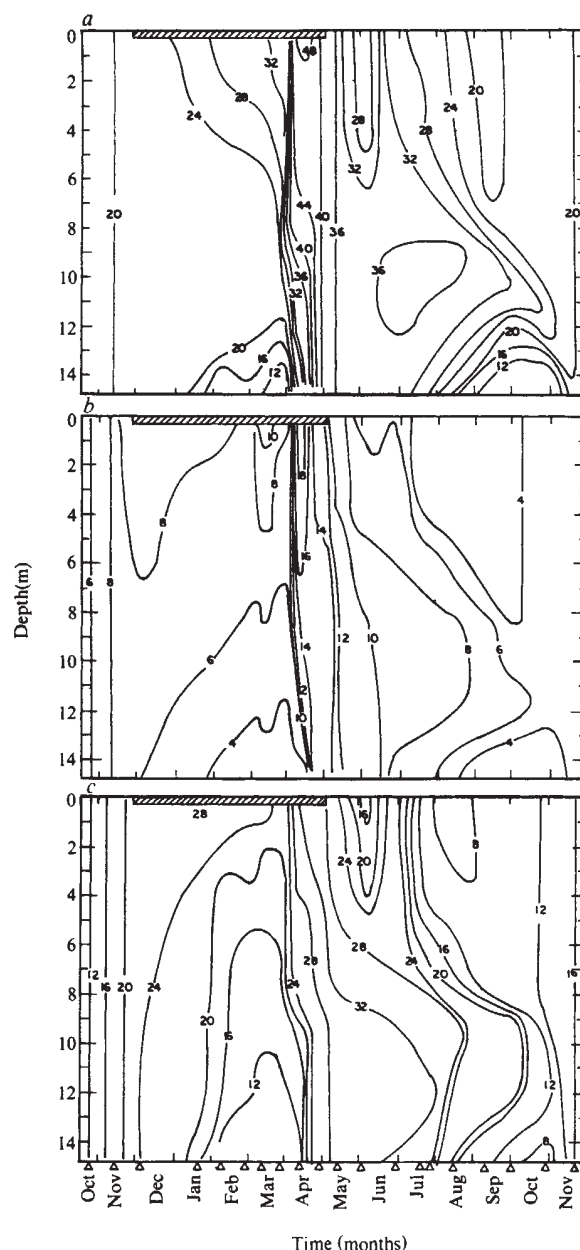


Fig. 1 Isopleths of nitrate (a), hydrogen ion BNC (b) and aluminium BNC (c) in $\mu\text{Eq l}^{-1}$ for Dart Lake (25 October 1981–21 November 1982). Samples were collected at seven depths. Sampling dates are indicated (Δ).

organic solutes in $\mu\text{mol l}^{-1}$ and DOC is in $\mu\text{mol l}^{-1}$; $n = 13$, $r^2 = 0.69$, $P < 0.01$). A relatively consistent fit to a proton dissociation constant was also obtained ($pK_a = 4.53 \pm 0.25$). Our detailed observations of DOC were applied to this model to estimate the BNC due to organic solutes and the organic anion concentration of water samples.

Poor precision, slow kinetics associated with heterogeneous phase reactions, and the inability to characterize individual components, meant that BNC was not determined by titration. Rather the individual components of BNC were calculated, using a chemical equilibrium model¹⁴, as the amount of strong base required to increase the pH of a litre of solution to a value of 8.3. The thermochemical data used in our analysis are summarized elsewhere^{11,12} and thermodynamic calculations were corrected for the effects of temperature and ionic strength. In these calculations we assumed that labile monomeric aluminium was a good estimate of inorganic forms of aluminium, and that non-labile monomeric aluminium did not significantly accept or donate protons. The close electroneutrality balance supports

Table 1 Mean chemical composition ($\mu\text{Equiv l}^{-1}$) of Dart Lake inlet, outlet and water column solutions, October 1981–November 1982 ($n = 172$)

	Mean	s.d.
Base neutralizing capacity (to reference $\text{pH} = 8.3$)		
Hydrogen ion	7.4	3.7
Aluminium	19.6	8.2
Carbonic acid	62.0	58.9
Organic acids	3.0	1.2
Total	92.0	59.0
Basic cations		
Calcium	99.5	4.6
Magnesium	28.9	2.2
Sodium	28.3	4.9
Potassium	13.0	1.1
Total	169.7	10.0
Anions		
Sulphate	137.6	11.0
Nitrate	24.4	8.6
Chloride	13.5	3.4
Organic anions	12.2	0.4
Bicarbonate	7.2	11.9
Free fluoride	0.9	0.7
Total	195.8	25.6
Sum of cations*	196.7	13.9
Sum of anions	195.8	25.6

* Cationic equivalences were computed to be hydrogen ion and aluminium BNC plus basic cations.

the assumptions and procedures used in this study for the characterization of aluminium and DOC (Table 1). Further information on the site description, hydrology, sampling and analytical methods, thermal and water quality data, and data analysis are available elsewhere¹³.

Although H_2CO_3 was the major component of BNC in Dart Lake (Table 1), levels of hydrogen ion and aluminium BNC in acid lakes are generally of more interest because of potential toxicity to organisms^{15,16}. Variations in hydrogen ion and aluminium BNC (H–Al–BNC) of all samples collected ($n = 178$) were strongly correlated with variations in nitrate concentration ($\text{H–Al–BNC} = 0.94 \text{ NO}_3^- + 2.4$; $\mu\text{Equiv l}^{-1}$, $r^2 = 0.54$, $P < 0.0001$). Note that this empirical correlation is linear with a slope close to one and an intercept near the origin. Although SO_4^{2-} was the dominant anion in Dart Lake (Table 1), no statistically significant relationship with H–Al–BNC was observed. H–Al–BNC was weakly correlated with organic anion concentration ($\text{H–Al–BNC} = 33.3 (\text{RCOO}^-) - 481$; where RCOO^- represents the organic anion content in $\mu\text{Equiv l}^{-1}$, $r^2 = 0.13$, $P < 0.0001$) and no statistically significant relationship was observed with Cl^- .

Water quality observations in Dart Lake suggest that biogeochemical processes result in temporal and spatial variations in NO_3^- , which influence hydrogen ion and aluminium BNC (Fig. 1). In the autumn, NO_3^- , H–BNC and Al–BNC exhibited an orthograde distribution in Dart Lake. During ice cover, NO_3^- , H–BNC and Al–BNC increased in the upper waters. These

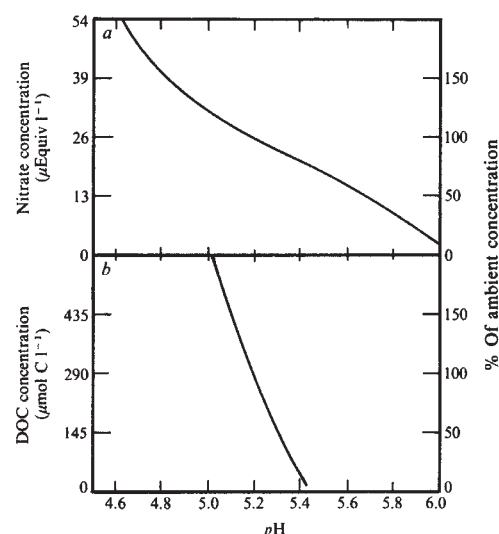


Fig. 2 Simulated changes in the pH of Dart Lake with increases and reductions from the ambient nitrate (a) and DOC (b) concentrations. Average Dart Lake water (Table 1) was assumed to be in equilibrium with microcrystalline gibbsite ($p^*K_{so} = 9.35$; ref. 28) and an organic solute ($C_T = 0.025 \text{ DOC} + 8.0$, in $\mu\text{mol l}^{-1}$; $pK_a = 4.53$). The relatively soluble mineral phase, microcrystalline gibbsite, was selected for our simulations because it is considered to represent the solubility of freshly-formed $\text{Al}(\text{OH})_3$ (ref. 23). Moreover, the mean ion activity (Q_{so}) product for $\text{Al}(\text{OH})_3$ of Dart Lake solutions was very close to the solubility of microcrystalline gibbsite ($p^*Q_{so} = 9.52 \pm 0.28$).

trends were presumably due to freeze-concentration at the ice–water interface and/or higher concentrations associated with stream inputs, which flow along the lake surface because of winter thermal stratification¹⁷. Concentrations of all three constituents decreased with increasing lake depth. Although the lake was aerobic throughout the study period (a minimum DO of $41 \mu\text{mol l}^{-1}$ was observed at 14 m depth on 1 October 1982), we attribute these lower water depletions of BNC to sediment reduction of NO_3^- and the resulting production of acid neutralizing capacity (ANC).

During the snowmelt period (1 March–1 May), 34% of the annual discharge entered Dart Lake. Inlet concentrations of H–Al–BNC and NO_3^- were significantly greater during snowmelt (1 March–1 May, flow = $8.9 \pm 6.9 \text{ m}^3 \text{ s}^{-1}$, H–Al–BNC = 52 ± 11 , $\text{NO}_3^- = 51 \pm 11$) than during either summer–autumn (1 June–1 November, flow = $1.7 \pm 1.7 \text{ m}^3 \text{ s}^{-1}$; H–Al–BNC = 18 ± 5 , $P < 0.05$ Student's t test; $\text{NO}_3^- = 24 \pm 5$, $P < 0.05$) or winter (1 November–1 March, flow = $1.5 \pm 1.0 \text{ m}^3 \text{ s}^{-1}$; H–Al–BNC = 23 ± 6 , $P < 0.05$; $\text{NO}_3^- = 26 \pm 6$, $P < 0.1$) base flow periods. These inputs resulted in high levels of NO_3^- , H–BNC and Al–BNC throughout Dart Lake before summer stratification (Fig. 1).

During summer stratification, NO_3^- , H–BNC and Al–BNC were depleted in the upper and lower waters of Dart Lake, resulting in a positive heterograde distribution. We attribute the upper water depletion to algal assimilation of NO_3^- (ref. 18) or littoral sediment denitrification and the lower water depletion to pelagic sediment denitrification. There have been reports that

Table 2 Average chemical composition ($\mu\text{Equiv l}^{-1}$) of low ionic strength waters from lake districts in eastern North America²⁰

Region	No. of samples	H^+	Ca^{2+}	SO_4^{2-}	NO_3^-	HCO_3^-	Ref.
Adirondack New York, USA	206	12	108	135	16	25	25
Experimental Lakes Area, Ontario, Canada	102	1	96	78	1	74	26
Sudbury, Ontario, Canada	208	4	273	252	2	160	27
Churchill Falls, Labrador, Canada	13	1	80	60	2	69	28
Kejimikujik, Nova Scotia, Canada	3	18	22	76	3	0	29

the net assimilation of nitrate results in the production of ANC and an increase in pH values^{19,20}. Groundwater inflow, enriched in ANC, basic cations (Ca^{2+} , Mg^{2+} , Na^+ , K^+), and dissolved silica from soil weathering reactions, could also deplete H-Al-BNC during summer low-flow conditions. However, we observed no statistically significant variations in the inlet concentrations of basic cations and dissolved silica over the study period, suggesting that base flow input of ANC was not a significant mechanism of H-Al-BNC neutralization. In the extreme lower waters (below 12 m, which is 5% of the lake volume) during stratification, transformations of Fe, Mn, DOC, Ca^{2+} , and NH_4^+ contributed to the depletion of H-BNC and Al-BNC in Dart Lake. However, on a whole-lake basis, nitrogen transformations affecting NO_3^- concentration seem to be the predominant mechanism regulating short-term changes in hydrogen ion and aluminium in solution.

The results of synoptic surveys indicate that NO_3^- levels are generally higher in the Adirondack lake district than other lake districts in eastern North America²¹ with low ionic strength waters (Table 2). A difficult but critical problem is to identify the source of this nitrate. Johannes *et al.*²² indicated that NO_3^- storage in snowpack from the Adirondack region is generally greater than SO_4^{2-} . Galloway *et al.*⁷ have attributed the decrease in the ANC that occurs in the Adirondack lake outlets during snowmelt, to a reduction in basic cation release from soils and the addition of nitric acid from snowpack. While snowpack nitrate undoubtedly contributes to the elevated levels observed in Adirondack lake waters, decomposition and oxidation of organic nitrogen within the adjacent soil may also result in the release of nitrate and BNC to solutions^{23,24}.

Because of these apparently high NO_3^- levels in Adirondack waters we wanted to evaluate the extent to which changes in ambient anion concentrations (SO_4^{2-} , NO_3^- , RCOO^-) result in changes in solution pH. To accomplish this, we simulated the response of Dart Lake water (Table 1) to additions and reductions in HNO_3 , H_2SO_4 , and the protonated form of naturally occurring organic solutes using a chemical equilibrium model¹⁴. Modest additions and depletions of NO_3^- resulted in significant changes in pH, while Dart Lake appears to be well buffered with respect to changes in DOC (Fig. 2). The latter response may be attributed to the relatively low density of proton dissociation sites per mol of organic carbon and indicates that deprotonation of organic acids probably has not contributed significantly to the acidification of Dart Lake. When NO_3^- levels were reduced to values comparable to other lake districts in eastern North America ($\sim 2 \mu\text{equiv l}^{-1}$) pH values approached neutrality. Like nitrate, variations in the concentration of H_2SO_4 result in an equivalent change in H-Al-BNC. Because SO_4^{2-} is the dominant anion in Dart Lake solutions (Table 1), neutralization can also be accomplished by a reduction in the concentration of H_2SO_4 . Note that in extremely acidic (pH < 4.8) Adirondack lakes, H-Al-BNC generally exceeds NO_3^- concentrations²². Therefore surface water acidification in the Adirondacks cannot be entirely attributed to HNO_3 , but more probably a combination of H_2SO_4 and HNO_3 inputs.

Although SO_4^{2-} is usually the dominant anion in Adirondack surface waters²⁵ and in atmospheric inputs to the region²⁴, additions of NO_3^- during snowmelt and depletions of NO_3^- associated with assimilation and reduction processes appear to be very important in regulating short term changes in hydrogen ion and aluminum concentrations in an acidic Adirondack lake. Moreover, elevated levels of NO_3^- in the Adirondack Lake district, compared with other lake districts in eastern North America, suggest that nitric acid inputs may have also contributed to the long term acidification of the region. However, a more complete understanding of the processes controlling the transport of NO_3^- and H-Al-BNC during snowmelt is essential to assess the mechanisms responsible for surface water acidification.

We thank L. G. Barnum, N. J. Peters, F. J. Unangst and J. R. White for assistance. This study was supported in part by the USEPA/NCSU Acid Deposition Program (APP 0094-1981).

This paper does not necessarily reflect the views of the EPA and no official endorsement should be inferred. Contribution no. 31 of the Upstate Freshwater Institute.

Received 12 October 1983; accepted 9 May 1984.

- Rosenqvist, I. T. Sur jord-surt vann, Ingenorforlaget, Oslo (1977).
- Driscoll, C. T. & Likens, G. E. *Tellus* **34**, 283-292 (1982).
- Kelly, C. A., Rudd, J. W. M., Cook, R. B. & Schindler, D. W. *Limnol. Oceanogr.* **27**, 868-882 (1982).
- van Breemen, N., Mulder, J. & Driscoll, C. T. *Plant Soil* **75**, 283-308 (1983).
- Gjessing, E. T., Henriksen, A., Johannessen, M. & Wright, R. F. in *Impact of Acid Precipitation on Forest and Freshwater Ecosystems in Norway* (ed. Braekke, F. H.) 64-85 (SNSF Project As, Norway, 1976).
- Henriksen, A. *Nature* **278**, 542-545 (1979).
- Galloway, J. N., Schofield, C. L., Hendrey, G. R., Peters, N. J. & Johannes, A. H. in *Ecological Impact of Acid Precipitation* (eds Drablos, D. & Tollan, A.) 264-265 (SNSF Project, As, Norway, 1980).
- Glover, G. M. & Webb, A. H. *Water Res.* **13**, 781-783 (1979).
- Driscoll, C. T. & Bisogni, J. J. in *Modeling of Total Acid Precipitation Impacts* (ed. Schnoor, J. L.) 53-72 (Ann Arbor, Ann Arbor, 1984).
- Barnes, R. B. *Chem. Geol.* **15**, 177-191 (1975).
- Driscoll, C. T. *Int. J. Envir. Analyt. Chem.* **16**, 67-284 (1984).
- Johnson, N. M., Driscoll, C. T., Eaton, J. S., Likens, G. E. & McDowell, W. M. *Geochim. cosmochim. Acta* **45**, 1421-1437 (1981).
- Driscoll, C. T. & Schafran, G. C. *Final Rep. USEPA/NCSU Acid Precipitation Program Project APP 0094-1981* (1984).
- Westall, J. C., Zachary, J. L. & Morel, F. M. M. *MINEQL, A Computer Program for the Calculation of Chemical Equilibrium in Aqueous Systems* (Civil Engineering Department, Massachusetts Institute of Technology, Tech. Note, No. 18, 1976).
- Leivestad, H., Hendrey, G., Muniz, I. P. & Snekvik, E. in *Impact of Acid Precipitation on Forest and Freshwater Ecosystems in Norway* (ed. Braekke, F. H.) 86-111 (SNSF Project As, Norway, 1976).
- Cronan, C. S. & Schofield, C. L. *Science* **204**, 304-305 (1979).
- Hagen, A. & Langeland, A. *Envir. Pollut.* **5**, 45-57 (1973).
- Brewer, P. G. & Goldman, J. C. *Limnol. Oceanogr.* **21**, 108-117 (1976).
- Harvey, H., Pierce, R. C., Dillon, P. J., Kramer, J. R. & Welpdale, D. M. *Acidification of the Canadian Aquatic Environment* (Publ. NRCC No. 18475, Environment Secretariat National Research Council of Canada, 1981).
- Dillon, P. J. & Scheider, W. A. in *Modeling of Total Acid Precipitation Impacts* (ed. Schnoor, J. L.) 121-154 (Ann Arbor, 1984).
- Wright, R. F. *Predicting Acidification of North American Lakes* (Norwegian Institute for Water Research, Oslo, 1983).
- Johannes, A. H., Galloway, J. N. & Troutman, D. E. in *Ecological Impact of Acid Precipitation* (eds Drablos, D. & Tollan, A.) 260-261 (SNSF Project, As, Norway, 1980).
- Hem, J. D. & Roberson, C. E. *U.S. geol. Surv. Wat. Supply Pap.* 1827-A (1967).
- Johannes, A. H. & Altwicker, E. R., in *Ecological Impact of Acid Precipitation* (eds Drablos, D. & Tollan, A.) 256-257 (SNSF Project, As, Norway, 1980).
- Schofield, C. L. *Acidification of Adirondack Lakes by Acid Precipitation: Extent and Magnitude of the Problem* (USFWS Federation Aid Fish Restoration Project F-28-R, 1976).
- Beamish, R. J., Blouw, L. M. & McFarlane, G. A. *A Fish and Chemical Study of 109 Lakes in the Experimental Lakes Area, Northwestern Ontario, with Appended Reports on Lake Whitefish Ageing Errors and the Northwestern Ontario Baitfish Industry* (Tech. Rep. 607, Fisheries and Marine Service Environment Canada, 1976).
- Conroy, N., Hawley, K. & Keller, W. *Extensive Monitoring of Lakes in the Greater Sudbury Area 1974-1976* (Ontario Ministry of the Environment, 1978).
- Duthie, H. C. & Ostrofsky, M. L. *J. Fish Res. Bd. Can.* **31**, 1105-1117 (1974).
- Kerekes, J. J. in *Ecological Impact of Acid Precipitation* (eds Drablos, D. & Tollan, A.) 232-233 (SNSF Project, As, Norway, 1980).

Interspecific competition is not a major organizing force in many insect communities

Bryan Shorrocks, Jon Rosewell & Kathy Edwards

Department of Pure and Applied Zoology, University of Leeds, Leeds LS2 9JT, UK

Will Atkinson

Department of Genetics and Human Variation, La Trobe University, Bundoora, Victoria, Australia 3083

Part of the current dogma in ecology is that competition between species for limited resources is not only common but also a major organizing force in many communities^{1,2} largely because studies on vertebrates, particularly birds, have played a major role in creating the traditional framework of niche theory and resource partitioning³⁻⁹. Other workers, particularly those studying insect communities, have suggested that significant interspecific competition is too rare and sporadic to be of major significance and have placed more emphasis on autecological processes¹⁰⁻¹³. Efforts to resolve the controversy have concentrated on the question of whether or not competition is common in nature¹. Here we show that even where competition can be demonstrated, it need not have a major role in community organization.

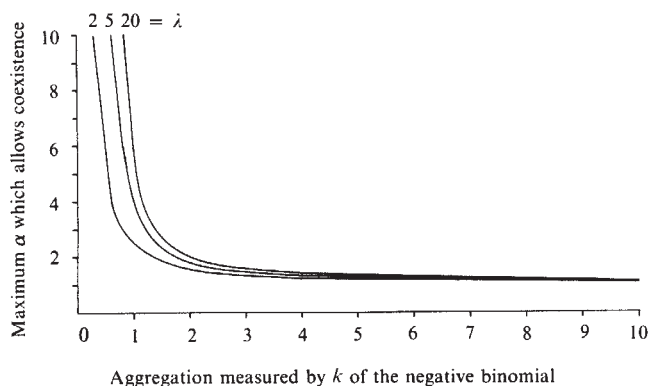


Fig. 1 Relationship between aggregation (k) and the maximum value for the competition coefficient (α) of the 'superior' species which allows coexistence. Within each site, competition is modelled for each species using the equations of Hassell and Comins¹⁶:

$$n_i(t+1) = \lambda_i n_i(t) [1 + a_i(n_i(t) + \alpha_{ij} n_j(t))]^{-b_i}, i \neq j$$

where $n_i(t)$ and $n_j(t)$ are the numbers of each species in a breeding site at time t , λ_i is the net reproductive rate, α_{ij} is a competition coefficient and a_i and b_i are constants. The parameter a_i is related to the population size per site at which density dependence starts to act and b_i describes the type of competition. As b_i increases, competition becomes less contest and more scramble¹⁸. In this figure we have chosen very stringent conditions for coexistence ($a = 0.4$, $b = 1$ and λ between 2 and 20 for both species, $\alpha = 0$ for the inferior species). Note that, unlike Atkinson and Shorrocks¹⁵, we have given a as expressed per site.

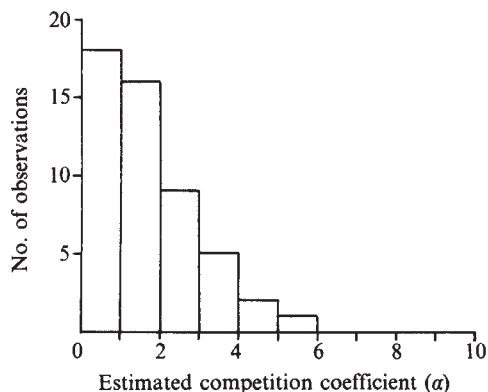


Fig. 2 Values of α taken from the literature for species of *Drosophila*¹⁹⁻²⁴. All values are measured in confined laboratory conditions and are probably maximum values. Species of *Drosophila* used are *D. busckii*, *D. melanogaster*, *D. nebulosa*, *D. pseudoobscura*, *D. serrata*, *D. simulans* and *D. willistoni*.

Our conclusions stem from work on wild *Drosophila* populations¹⁴, in which the population dynamics have been modelled by Atkinson and Shorrocks¹⁵. The model applies to species exploiting discrete and ephemeral breeding sites such as dung, carrion, sap flows, fallen fruit or fungi. It shows that two species might never exclude one another globally, despite strong local competition between the larvae if the competing stages are aggregated. In the model, each species distributes its eggs over the breeding sites according to a negative binomial distribution, which has an exponent, k , inversely related to the degree of aggregation. Within each site competition is modelled using the competition equations of Hassell and Comins¹⁶ in which the competition coefficients α measure the effect of an individual of one species on the other.

The model's conclusions (which have been confirmed analytically¹⁷) are summarized in Fig. 1. As the degree of aggregation

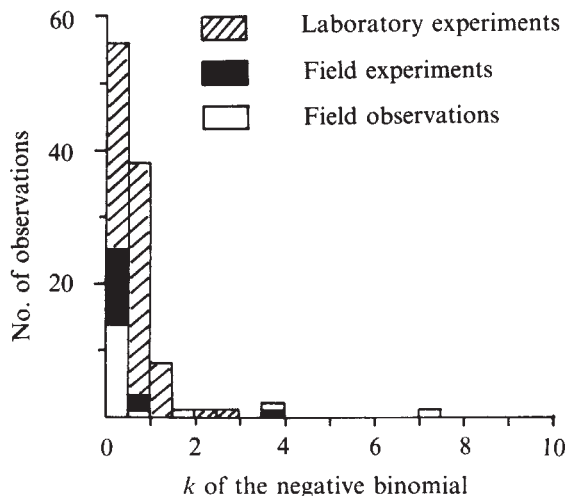


Fig. 3 k of the negative binomial for the distribution of 23 dipteran species in three different conditions—field observations²⁵, field experiments (J.R., unpublished data) and laboratory experiments (ref. 25 and B.S. and K.E., unpublished data). There is no significant difference between the values of k obtained from the three types of measurement ($\chi^2 = 4.13$). The laboratory experiments used *D. immigrans* and *D. melanogaster*, with 4 cm² and 9 cm² units of laboratory *Drosophila* medium as oviposition sites. The field experiments gave data for *D. immigrans*, *D. melanogaster* and *D. subobscura*, with 4 cm² units of apple, lemon, onion and potato as oviposition sites. The field observations came from eight species of Australian fruit (536 items) and used *D. buzzatii*, *D. fumida*, *D. immigrans*, *D. lativitta*, *D. pseudotakahashii*, *D. simulans*, *D. spaciensis*. Additional dipteran species came from the Anisopodidae, Chironomidae, Chloropidae, Sphaeroceridae, Muscidae, Psychodidae, Ceratopogonidae and Empididae.

increases, it becomes harder for the superior species to exclude the other. Moreover, given the typical values of α in Fig. 2, it is obvious that when the potential competitors are highly aggregated ($k < 1$), global competitive exclusion is virtually impossible. We have measured k of the negative binomial for the immature stages of 23 dipteran species including 12 *Drosophila* and the results are summarized in Fig. 3. Of the 108 (32 field) values of k , 94 (28 field) are less than 1.

The conclusion from combining the parameter values in Figs 2 and 3 is that *Drosophila* species will rarely exclude one another in nature even if they show no traditional resource partitioning and compete most strongly. The most important parameter determining global coexistence is not α , the local competition coefficient, but k , which is a spatial parameter. Furthermore, if the superior competitor is removed from our computer simulation, the competitively inferior species responds by increasing its numbers. Thus, the removal experiments collected by Schoener¹, although providing evidence for the presence of competition in natural communities, do not necessarily imply that competition is present and is a major organizing force in community structure.

Received 16 March; accepted 15 May 1984.

1. Schoener, T. W. *Am. Nat.* **122**, 240–285 (1983).
2. Roughgarden, J. *Am. Nat.* **122**, 583–601 (1983).
3. Cody, M. L. *Competition and the Structure of Bird Communities* (Princeton University Press, 1974).
4. Diamond, J. M. in *Ecology and Evolution of Communities* (eds Cody, M. L. & Diamond, J. M.) 342–444 (Harvard University Press, 1975).
5. Diamond, J. M. *Am. Sci.* **66**, 322–331 (1978).
6. Lack, D. *Ecological Isolation in Birds* (Harvard University Press, 1971).
7. MacArthur, R. H. in *Avian Biology Vol. 1* (Academic, New York, 1971).
8. Pianka, E. E. in *Ecology and Evolution of Communities* (eds Cody, M. L. & Diamond, J. M.) 292–314 (Harvard University Press, 1975).
9. Roughgarden, J., Heckel, D. & Fuentes, E. in *Lizard Ecology* (eds Huey, R. B., Pianka, E. R. & Schoener, T. W.) 371–410 (Harvard University Press, 1983).
10. Andrewartha, H. G. & Birch, L. C. *The Distribution and Abundance of Animals* (Chicago University Press, 1954).

11. Lawton, J. H. & Strong, D. R. *Am. Nat.* **118**, 317–338 (1981).
12. Strong, D. R. *Am. Nat.* **122**, 636–660 (1983).
13. Connell, J. H. in *Ecology and Evolution of Communities* (eds Cody, M. L. & Diamond, J. M.) 460–490 (Harvard University Press, 1975).
14. Shorrock, B. in *Genetics and Biology of Drosophila* Vol. 3b (eds Ashburner, M., Carson, H. L. & Thompson, J. N. Jr) 385–428 (Academic, London, 1982).
15. Atkinson, W. D. & Shorrock, B. *J. Anim. Ecol.* **50**, 461–471 (1981).
16. Hassell, M. P. & Comins, H. N. *Theor. Pop. Biol.* **9**, 202–221 (1976).
17. May, R. M. & Ives, A. R. *J. theor. Biol.* (in the press).
18. Nicholson, A. J. *Aust. J. Zool.* **2**, 9–65 (1965).
19. Ayala, F. J. *Nature* **224**, 1076–1079 (1969).
20. Ayala, F. J. in *Essays in Evolution and Genetics in Honor of Theodosius Dobzhansky* (eds Hecht, M. K. & Steere, W. C.) 121–158 (Appleton-Century-Crofts, New York, 1970).
21. Ayala, F. J. *Am. Scient.* **60**, 348–357 (1972).
22. Ayala, F. J., Gilpin, M. E. & Ehrenfeld, J. G. *Theor. Pop. Biol.* **4**, 331–356 (1973).
23. DeBenedictis, P. A. *Ecology* **58**, 158–166 (1977).
24. Miller, R. S. *Ecology* **45**, 132–148 (1964).
25. Atkinson, W. D. & Shorrock, B. *Am. Nat.* (in the press).

Origin of a gene regulatory mechanism in the evolution of echinoderms

Rudolf A. Raff, John A. Anstrom, Carolyn J. Huffman, David S. Leaf, Jun-Hun Loo, Richard M. Showman* & Dan E. Wells*

Institute for Molecular and Cellular Biology, and Department of Biology, Indiana University, Bloomington, Indiana 47405, USA

A rich diversity of ancient sea urchin lineages survives to the present. These include several advanced orders as well as the cidaroids, which represent the group ancestral to all other sea urchins. Here we show that all advanced groups of sea urchins examined possess in their eggs a class of maternal messenger RNA (mRNA) encoded by the evolutionarily highly conserved α -subtype histone genes. The maternal histone mRNAs are unique in their time of accumulation in oogenesis, their localization in the egg nucleus and their delayed timing of translation after fertilization. Cidaroid sea urchins as well as other echinoderm classes, such as starfish and sea cucumbers, possess the genes but do not have maternal α -subtype histone mRNAs in their eggs. Thus, although all the echinoderms examined transcribe α -subtype histone genes during embryogenesis, the expression of these genes as maternal mRNAs is confined to advanced sea urchins. The fossil record allows us to pinpoint the evolution of this mode of expression of α -histone genes to the time of the splitting of advanced sea urchin lineages from the ancestral cidaroids in a radiation which occurred in a relatively brief interval of time ~190–200 Myr ago. The origin of a unique gene regulatory mechanism can thus be correlated with a set of macroevolutionary events.

A modern phylogeny¹ of the major groups of sea urchins is presented in Fig. 1. There was a dramatic and rapid radiation of new echinoid orders in the last 5–10 Myr of the Triassic². The major events were the origin of the living cidaroids (Cidaridae), the diademids (Diadematacea) and the advanced regular echinoids (Echinacea). The diademids gave rise to a complex group of irregular sea urchins in the Jurassic. The Echinacea include three orders which contain genera frequently used for embryological and molecular investigations: *Arbacia* (order Arbacioida); *Lytechinus* (order Temnopleurida); and *Paracentrotus*, *Psammechinus* and *Strongylocentrotus* (order Echinoida). Furthermore, most old orders of sea urchins still survive. Thus, molecular phylogenetic studies can be carried out on echinoid groups of various evolutionary ages which have readily available living representatives and reasonably well-defined evolutionary relationships based on the fossil record.

Sea urchins possess several families of developmentally regulated histone genes^{3–5}. During development, the temporal sequence of synthesis of histones begins with cs-subtype (cleavage stage) histones synthesized from fertilization for the

first few cleavages. This is followed by massive synthesis of α -subtype histones until the blastula stage when α -histone synthesis ends, giving way to synthesis of late subtypes. There are distinct, separate sets of genes for each of the subtypes^{6,7}.

We examined the histone genes and their patterns of expression in a variety of echinoderm species to compare the use of a gene set among different orders of known evolutionary relationships. We used labelled probes prepared from clones of individual α -histone-coding sequences from *Strongylocentrotus purpuratus*. We first established that the genes in all echinoderms examined are, in fact, both evolutionarily homologous and conserved. Two criteria were used: DNA sequence conservation and presence of topologically similar tandem gene clusters.

One might expect the histone genes of species that are separated by a long evolutionary period to be highly diverged⁸. Instead, we found that hybrids between H3 genes of *S. purpuratus* with DNA of the cidaroid *Eucidaris tribuloides* (diverged 200 Myr ago) or the starfish *Asterias forbesi* (diverged >500 Myr ago) are only about 12% divergent in sequence. This degree of divergence is similar to that between H3 or H4 genes of *Lytechinus* and *Strongylocentrotus* whose lineages split ~80 Myr ago (ref. 8 and our unpublished data). This effect is similar to that observed in preproinsulin and globin genes⁹, and implies that the genes are under potent selective constraints for both nucleotide and protein sequence. This had the practical consequence that there was strong hybridization of probes to all species used.

The α -histone gene cluster has been cloned and mapped for several sea urchins of the orders Echinoida and Temnopleurida^{4,5}. We have determined the organization of the core histone genes in the clusters of two phylogenetically important species (Fig. 2). The starfish *A. forbesi* was used as a representative of a non-echinoid echinoderm class, while *E. tribuloides* was taken as a representative of the cidaroid root stock of all modern echinoids. Both species have tandemly repeated clusters (on the order of a few hundred) of α -histone genes. The topology of the *E. tribuloides* cluster is like that of *S. purpuratus*. The starfish *A. forbesi* has a similar α -histone gene cluster but with a somewhat different topology (the H3 gene lies between the H2B and H4 genes; Fig. 2).

Expression of α -histone genes has been well defined for the sea urchin *S. purpuratus* (order Echinoida; Fig. 3a). The unfertilized egg of *S. purpuratus* contains a complex set of mRNA species, each present in a few thousand copies per egg, which are synthesized and accumulate throughout oogenesis^{10,11}. These mRNAs are present in the egg cytoplasm as mRNPs and remain almost entirely untranslated in the egg, which exhibits only a slow rate of protein synthesis. Immediately after fertilization, maternal mRNAs begin to enter polysomes and are translated^{12,13}. In contrast, the α -histone mRNAs do not enter polysomes until 1.5–2 h post-fertilization, at the first mitotic cleavage^{13,14}. This temporal delay in α -histone mRNA recruitment is linked to a unique localization of maternal α -histone mRNAs in the female pronucleus of the egg^{14–17}. These mRNAs are retained in the nucleus after fertilization until the time of breakdown of the nuclear membrane at first cleavage. The α -histone mRNAs, unlike other maternal mRNAs, accumulate in eggs only after the completion of meiosis¹⁸. *Arbacia punctulata* (order Arbacioida) and *Lytechinus pictus* (order Temnopleurida) exhibit similar behaviour¹⁹. Representatives of two other distinct major groups of sea urchins (shown in Fig. 1) also store maternal α -histone mRNAs—these are *Diadema antillarum* (order Diademataceae; data not shown) and the sand dollars *Dendraster excentricus*, *Clypeaster subdepressus* and *Echinarachnius parma* (all order Clypeasteroida; Fig. 3d).

Figure 3c shows that, like advanced sea urchins^{20,21}, *E. tribuloides* embryos transcribe the α -histone genes for a few hours during cleavage, with a peak of mRNA accumulation at 9 h, but, unlike the other major groups of sea urchins, α -histone mRNAs are absent from eggs.

As the major sea urchin lineages have been distinct for about 190 Myr, it seems that the regulatory mechanisms responsible

* Present addresses: Department of Biology, University of South Carolina, Columbia, South Carolina 29208, U.S.A. (R.M.S.); Veterans Administration Hospital, Miranda Avenue, Palo Alto, California 94305, U.S.A. (D.E.W.).

We thank M. Harkey, D. McClay and C. Simerly for providing fixed eggs and embryos, and R. Angerer, L. Kedes and E. Weinberg for providing clones. We also thank J. W. Durham and P. M. Kier for discussions of sea urchin phylogeny, and M. Smith for unpublished data. Grant support was from the NIH. D.S.L. was supported by a NIH predoctoral training grant.

Received 23 January; accepted 14 May 1984.

- Smith, A. B. *Palaeontology* **24**, 779–801 (1981).
- Kier, P. M. *Smithson. Contr. Paleobiol.* **30**, 1–89 (1977).
- Childs, G., Maxson, R. & Kedes, L. H. *Dev. Biol.* **73**, 153–173 (1979).
- Kedes, L. H. *A. Rev. Biochem.* **48**, 837–870 (1979).
- Hentschel, C. C. & Birnstiel, M. L. *Cell* **25**, 301–313 (1981).
- Childs, G., Nocente-McGrath, C., Lieber, T., Holt, C. & Knowles, J. A. *Cell* **31**, 383–393 (1982).
- Maxson, R., Mohun, T., Gormezano, G., Childs, G. & Kedes, L. *Nature* **301**, 120–125 (1983).
- Busslinger, M., Rusconi, S. & Birnstiel, M. L. *EMBO J.* **1**, 27–33 (1982).
- Perler, F. *et al. Cell* **20**, 555–566 (1980).
- Hough-Evans, B. R., Ernst, S. G., Britten, R. J. & Davidson, E. H. *Dev. Biol.* **69**, 258–269 (1979).
- Hough-Evans, B. R., Wold, B. J., Ernst, S. G., Britten, R. J. & Davidson, E. H. *Dev. Biol.* **60**, 258–277 (1977).
- Raff, R. A., Brandis, J. W., Huffman, C. J., Koch, A. L. & Leister, D. E. *Dev. Biol.* **86**, 265–271 (1981).
- Wells, D. E., Showman, R. M., Klein, W. H. & Raff, R. A. *Nature* **292**, 477–478 (1981).
- Showman, R. M., Wells, D. E., Anstrom, J., Hursh, D. A. & Raff, R. A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5944–5947 (1982).
- Venezky, D. L., Angerer, L. M. & Angerer, R. C. *Cell* **24**, 385–391 (1981).
- Raff, R. A. in *Time, Space, and Pattern in Embryonic Development* (eds Jeffery, W. R. & Raff, R. A.) (Liss, New York, 1983).
- DeLeon, D. V., Hughes, K. J., Angerer, L. M. & Angerer, R. C. *Dev. Biol.* **100**, 197–206 (1983).
- Angerer, L. M. *et al. Dev. Biol.* **102**, 477–484 (1984).
- Showman, R. M. *et al. in Molecular Aspects of Early Development* (eds Malacinski, G. M. & Klein, W. H.) (Plenum, New York, 1984).
- Mauron, A., Kedes, L., Hough-Evans, B. R. & Davidson, E. H. *Dev. Biol.* **94**, 425–434 (1982).
- Maxson, R. E. Jr & Wilt, F. H. *Dev. Biol.* **94**, 435–440 (1982).
- Paul, C. R. C. in *Patterns of Evolution* (ed. Hallam, A.) (Elsevier, Amsterdam, 1977).
- Ohama, T., Hori, H. & Osawa, S. *Nucleic Acids Res.* **11**, 5181–5184 (1983).
- Raff, R. A. & Kaufman, T. C. *Embryos, Genes, and Evolution* (Macmillan, New York, 1983).
- Schroeder, T. E. *Biol. Bull.* **161**, 141–151 (1981).
- Bruskin, A. M., Tyner, A. L., Wells, D. E., Showman, R. M. & Klein, W. H. *Dev. Biol.* **87**, 308–318 (1981).
- Sures, I., Lowry, J. & Kedes, L. H. *Cell* **15**, 1033–1044 (1978).
- Overton, G. C. & Weinberg, E. S. *Cell* **14**, 247–257 (1978).

Retinal light adaptation—evidence for a feedback mechanism

D. Tranchina*†, J. Gordon*‡ & R. M. Shapley*

*The Rockefeller University, New York, New York 10021, USA

†Courant Institute of Mathematical Sciences, New York University, New York, New York 10012, USA

‡Hunter College, City University of New York, New York, New York 10021, USA

Light adaptation is the adjustment of retinal response properties to variations in ambient illumination. It enables the encoding of visual information over a millionfold intensity range, from moonlight to broad daylight, despite the relatively small dynamic range of response of visual neurones. We have studied the effects of light adaptation on the dynamics and sensitivity of visual responses of neurones in the turtle retina, by measuring the responses of horizontal cells in the retina to light which was modulated with a sinusoidal time course around various mean levels. As a quantitative measure of the transduction from light to neural signals, we calculated the gain of response at each frequency. Gain is defined as the amplitude of the modulated response component divided by the amplitude of light modulation. We report here that the gain (mV photon^{-1}) at low temporal frequencies decreased as the mean light level increased. Over a 2 log-unit range of mean light levels, low-frequency gain was inversely proportional to the mean light level, as in Weber's law. However, at high temporal frequencies, the gain was almost independent of mean light level. Our results are reminiscent of Kelly's¹ results on human temporal-frequency sensitivity in various states of light adaptation. We found that a family of horizontal-cell temporal frequency responses, measured at various mean light levels, could be accounted for by a negative feedback model in which the feedback strength is proportional to mean light level.

Recent physiological experiments indicate a hierarchy of retinal adaptation mechanisms at various sites within the retina². In the turtle retina, one site of adaptation is located in individual cones^{3,4}. Here we analyse light adaptation in luminosity horizontal cells of the turtle retina; these cells receive synaptic input almost exclusively from 630 nm cones^{5–9} and make feedback synapses with these cones^{10,11}. Turtle horizontal cells respond approximately linearly to modulations of illuminance around a mean level^{12,13}. Therefore, their response properties, at any given mean level, can be characterized by a temporal frequency response. A good measure of light adaptation is change in the temporal frequency response as the mean light level varies.

The physiological preparation, and most of the materials and methods used, have been described previously¹⁴. Intracellular recordings were obtained from luminosity horizontal cells in eye-cup preparations of *Pseudemys scripta elegans* (nine cells) and *Chelydra serpentina* (three cells).

Figure 1 shows frequency responses measured at five mean light levels spanning a range of 4 log-units; response gain and phase are plotted in *a* and *b*, respectively. The symbols represent experimental data, and the curves are given by the feedback model described below. The uppermost gain curve corresponds to the lowest mean light level. As the mean light level increases, the gain of low-frequency responses decreases. Over a range of high mean light levels, the gain at low frequencies is inversely proportional to the mean; this behaviour is a physiological analogue of Weber's psychophysical law. It implies that, over a range of high light levels, the amplitude of a low-frequency response is constant for a fixed contrast of stimulus (see, for example, ref. 2). High-frequency responses, however, behaved in an entirely different manner. The gain at high frequencies was approximately independent of mean light level; the family of gain curves tends towards a common high-frequency asymptote.

The family of phase curves shows, at the lowest modulation frequency (0.125 Hz), a phase near zero at all mean levels. At an intermediate frequency, the various phase curves are distinctly separated. The phase lag was greatest for the lowest mean light level. This observation is consistent with the qualitative notion that greater phase lag corresponds to a slower response at lower mean light levels. However, the phase curves converge again at high frequencies, as do the gain curves (Fig. 1). The convergence of the gain curves to a common high-frequency asymptote and the dependence of low-frequency gain on mean light level are suggestive of a feedback system in which the strength of feedback depends on mean light level and in which the feedback dies out at high frequencies.

The frequency responses in Fig. 1 can be accounted for in detail by the simple feedback model depicted schematically in Fig. 2. The response of the system is determined by a feed-forward and a feedback signal. The dynamics of feed-forward and feedback are characterized by the frequency responses $A(f)$ and $B(f)$, respectively, where f is temporal frequency. $A(f)$ and $B(f)$ do not depend on the mean light level, I_0 . The critical feature of this model is that the output of the feedback filter is multiplied by the mean light level. The frequency response, $H(f, I_0)$, of this model is given by

$$H(f, I_0) = \frac{A(f)}{1 + I_0 B(f)} \quad (1)$$

The salient consequences of equation (1) are as follows: (1) When the mean light level, I_0 , is low, $H(f, I_0)$ is approximately given by $A(f)$, independent of I_0 because the term $I_0 B(f)$ is small compared with 1. In other words, $A(f)$ is the low-light limit of the temporal frequency response. The –3 log-unit gain curve is close to the –4 log-unit curve (Fig. 1) because $I_0 B(f)$ is still small compared with 1 at the –3 log-unit level. (2) When the frequency f is large, the frequency response $H(f, I_0)$ is again approximately given by $A(f)$, independent of I_0 . This is because, at high frequencies, $B(f)$ is small, and the term $I_0 B(f)$ is small

compared with 1, even for moderately large I_0 . Figure 1 reflects this feature by the fact that the family of gain curves tends towards a common high-frequency asymptote. (3) At low frequencies, and high mean light levels, gain is inversely proportional to mean light level. This is because, in these conditions,

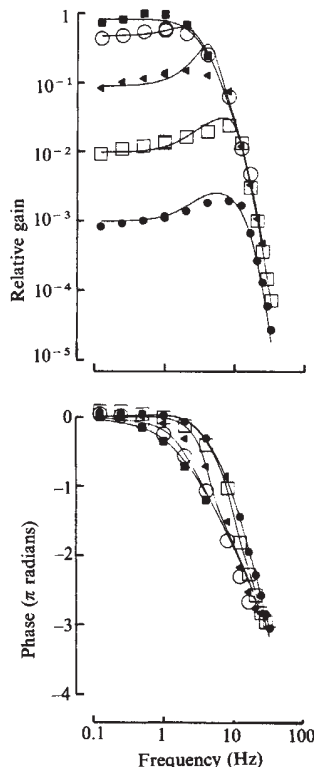


Fig. 1 A family of temporal frequency responses measured at mean light levels of -4 log units (■), -3 log units (○), -2 log units (▲), -1 log unit (□) and 0 log units (●). Symbols plot experimental data, and the continuous lines are given by equation (1) of the feedback model (Fig. 2). *a*, The relative response gain is plotted as a function of temporal frequency on log-log coordinates. *b*, The phase of the response is plotted as a function of temporal frequency on linear-log coordinates. Corresponding gain and phase for a given mean light level are plotted with the same symbol. The light stimulus was of the form $I(t) = I_0 + I_1 \sin(2\pi ft)$, where t is time, f is frequency in Hz, I_0 is the mean light level and I_1 is the amplitude of the sinusoidal component. The response was approximately of the form^{12,14} $R(t) = R_0 + R_1 \sin(2\pi ft + \theta)$. The gain of the response, $g(f, I_0)$, is given by $R_1(f, I_0)/I_1$, and $\theta(f, I_0)$ is the measured phase of the response. Relative response gain is defined as $g(f, I_0)/g_{\max}$, where $g_{\max}$ is the largest gain measured over all frequencies and mean light levels. The temporal frequency response, $H(f, I_0)$, is defined by $H(f, I_0) = g(f, I_0) \exp[i\theta(f, I_0)]$. The continuous lines were computed from equation (1) of the feedback model using the frequency responses $A(f)$ and $B(f)$ that are plotted with continuous lines in Fig. 3. The deviation of the model from the data (▲) at a frequency of 4 Hz and at the -2 log-unit mean light level is a common feature of several other data sets. The simple feedback model proposed here will have to be modified to give agreement between theory and experiment at this frequency and mean light level.

Methods: The light source used to measure frequency responses was a red light-emitting diode (LED; Stanley Hi-Super Brite, H500). The LED was placed behind a diffuser ~ 2 inches from the retina. Its intensity (mean and modulating component) was controlled by a servo circuit, in which the command signal was provided by a microcomputer, and the intensity of the LED's light was monitored by a photomultiplier. The irradiance produced by the LED in the retinal plane was measured with a spectrophotometer (Photo Research 1980b). The spectral peak of the light was at 670 nm. The density of photons falling on the retinal plane, at the highest mean light level used in this study (referred to as the 0 log-unit level) was 2.3×10^{13} photons per cm^2 , and 90% of these photons were in the 640 – 690 nm range.

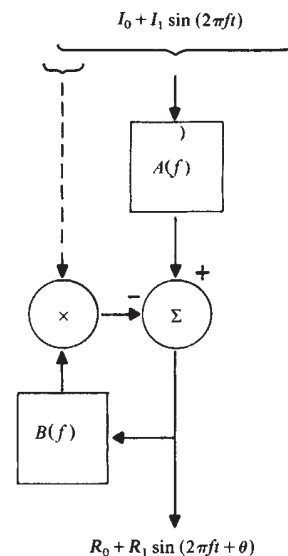


Fig. 2 Schematic diagram of the feedback model used to fit the data in Fig. 1. This circuit describes how the modulated component of spatially uniform light is transformed by the cone-horizontal cell network when the mean light level is I_0 . The circuit comprises two transducers (represented by the boxes). The frequency responses of the feed-forward and feedback transducers are $A(f)$ and $B(f)$, respectively. The gain of feedback is adjustable and is proportional to the mean light level. The adjustable feedback gain is denoted in the figure by a multiplication of the output of the feedback transducer by the mean light level, I_0 . The frequency response of this model is given by equation (1).

the term $I_0 B(f)$ is large compared with 1, and the frequency response $H(f, I_0)$ is approximately given by $(1/I_0)A(f)/B(f)$. Figure 3 shows the frequency responses $A(f)$ (*a*, *b*) and $B(f)$ (*c*, *d*) which gave the best fit of equation (1) to the experimental frequency responses in Fig. 1.

The fact that the feedback model accounts well for the data in Fig. 1 implies that the mechanism underlying light adaptation in turtle horizontal cells is equivalent to feedback whose strength is adjusted to be proportional to the level of illumination. It is logical to ask what is the physical nature of this feedback. We believe it is not neural feedback from horizontal cells to cones, principally because our preliminary measurements in cones show that the feedback model accounts for light adaptation even in conditions where neural feedback has been minimized. Therefore, light adaptation in turtle horizontal cells probably reflects chemical feedback within the phototransduction process in cones¹⁵.

If this hypothesis is correct, then any model for phototransduction in cones can be tested by determining whether it gives a frequency response which is approximately of the form shown in equation (1). A previous model for light adaptation in turtle cones¹⁵ is inconsistent with our data and with our model because it does not give Weber's law at low frequencies and high light levels². Ours is the simplest model which accounts for the major features of light adaptation in the outer plexiform layer of the turtle retina.

Our family of temporal frequency responses (Fig. 1) is similar to Kelly's¹ family of human temporal-frequency sensitivity curves measured psychophysically at various states of light adaptation. Chappell *et al.*¹⁶ studied light adaptation in turtle horizontal cells using techniques of Wiener analysis; they obtained results consistent with ours and have also noted the similarity between data for horizontal cells and Kelly's psychophysical data.

We acknowledge the helpful advice and insight of Charles Peskin and Bruce Knight. We thank Michelangelo Rossetto for designing and building the LED stimulator device, and Norman Milkman and Gary Schick for designing, building and maintain-

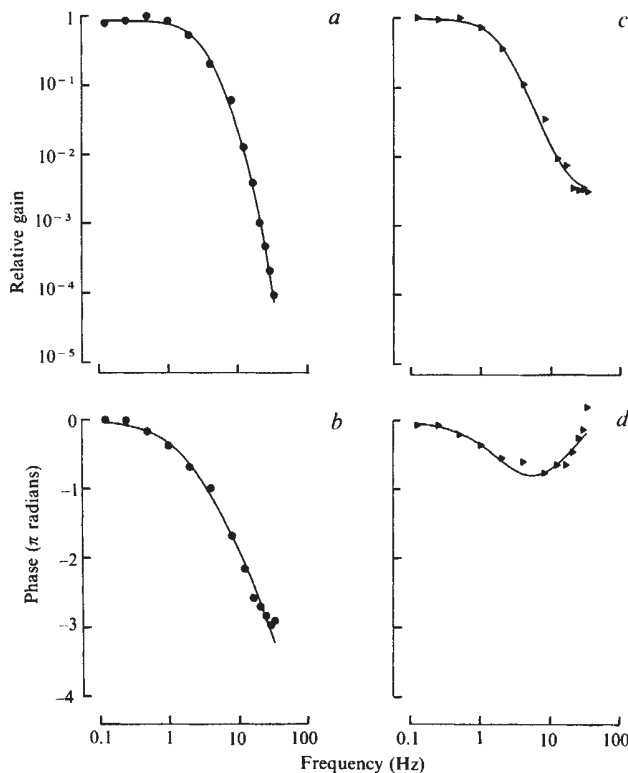


Fig. 3 *a, b*, Temporal frequency response of the feed-forward pathway $A(f)$ in the feedback model (see Fig. 2). The values of gain (*a*) and phase (*b*) which gave the best fit of equation (1) to the data in Fig. 1 are plotted with solid symbols. The continuous lines are given by a multi-stage low-pass filter with a frequency response of the form $A(f) = A_0(1 + i2\pi f\tau_1)^{-3}(1 + i2\pi f\tau_2)^{-7}$, where $\tau_1 = 46$ ms and $\tau_2 = 5.4$ ms. The absolute magnitude of A_0 was $\sim 4 \times 10^{-10}$ mV s cm² per photon. *c, d*, Frequency response of the feedback loop (open loop, when $I_0 = 1$). Best-fit values of amplitude (*c*) and phase (*d*) are plotted with solid symbols. Continuous lines are given by a multi-stage filter with a frequency response of the form $B(f) = B_0(1 + i2\pi f\tau_1)(1 + 2\pi f\tau_2)^3(1 + 2\pi f\tau_3)^{-1}(1 + i2\pi f\tau_4)^{-3}$, where $\tau_1 = 12.7$ ms, $\tau_2 = 6.8$ ms, $\tau_3 = 0.4$ ms, and $\tau_4 = 76.3$ ms. The value of $I_0 B_0$ was ~ 900 at the 0 log-unit mean light level. The best-fit values of the frequency responses $A(f)$ and $B(f)$ were determined by fitting equation (1) to the data (Fig. 1) using a least-squares method at each frequency separately.

ing much of the electronic equipment used. This work was supported by grants EY 1472 and EY 188 from the National Eye Institute. D.T. was supported by NSF grant MCS-8117526. Computer time was provided in part by the City University of New York-University Computer Center System.

Received 26 March; accepted 30 April 1984.

- Kelly, D. H. *J. opt. Soc. Am.* **51**, 747–754 (1961); **61**, 537–546 (1971).
- Shapley, R. M. & Enroth-Cugell, C. in *Progress in Retinal Research* (eds Osborne, N. & Chader, G.) (Pergamon, London, 1984).
- Baylor, D. A. & Hodgkin, A. L. *J. Physiol., Lond.* **242**, 729–758 (1974).
- Normann, R. A. & Perlman, I. *J. Physiol., Lond.* **286**, 491–508 (1979).
- Simon, E. J. *J. Physiol., Lond.* **230**, 199–211 (1973).
- Fuortes, M. G. F. & Simon, E. J. *J. Physiol., Lond.* **240**, 177–198 (1974).
- Yazulla, S. *Vision Res.* **16**, 727–735 (1976).
- Perlman, I. & Normann, R. A. *Vision Res.* **19**, 903–906 (1979).
- Fuortes, M. G. F., Schwartz, E. A. & Simon, E. J. *J. Physiol., Lond.* **234**, 199–216 (1973).
- Baylor, D. A., Fuortes, M. G. F. & O'Bryan, P. M. *J. Physiol., Lond.* **214**, 265–294 (1971).
- O'Bryan, P. M. *J. Physiol., Lond.* **235**, 207–223 (1973).
- Tranchina, D., Gordon, J., Shapley, R. & Toyoda, J.-I. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6540–6542 (1981).
- Naka, K.-I., Sakuranaga, M. & Chappell, R. L. *Biomed. Res.* **3**, Suppl., 131–136 (1982).
- Tranchina, D., Gordon, J. & Shapley, R. *J. gen. Physiol.* **82**, 573–598 (1983).
- Baylor, D. A., Hodgkin, A. L. & Lamb, T. D. *J. Physiol., Lond.* **242**, 759–791 (1974).
- Chappell, R. L., Sakuranaga, M. & Naka, K.-I. *Soc. Neurosci. Abstr.* **9**, 687 (1983).

How a gap junction maintains its structure

Jochen Braun, James R. Abney & John C. Owicki

Department of Biophysics and Medical Physics, University of California, and Division of Biology and Medicine, Lawrence Berkeley Laboratory, Berkeley, California 94720, USA

In gap junctions, identical membrane proteins are linked up in pairs (dyads) that bridge the extracellular space between two apposed cell membranes^{1,2}. Typically, several thousand of these dyads are aggregated in the plane of the membranes and form a junctional plaque with a distinct boundary. The question thus arises as to what maintains the dyads in an aggregated state. From a statistical mechanical analysis of the positions of dyads in a freeze-fracture electron micrograph, we report here that the aggregates are not maintained by an attractive force between pairs of dyads, but probably by the minimization of the repulsive force between apposed membranes. On the basis of this analysis we present a model for the structure of mature gap junctions as well as certain aspects of the formation and disassembly of gap junctions.

Freeze-fracture electron microscopy of tissue that has been rapidly frozen by the method of Heuser and Reese³ reveals to a good approximation the *in vivo* distribution of intramembrane particles⁴. Figure 1 shows a part of the electron micrograph of a gap junction in mouse liver that forms the basis of our analysis. As there is strong evidence against cytoskeletal attachment to dyads^{5–7}, it is plausible to assume that dyads are free to move laterally in the two junctional membranes and that aggregation into plaques is an equilibrium condition that reflects attractive forces among dyads. But before such a 'fluid mosaic' model can be satisfactory, the nature of the forces that maintain the aggregated state must be elucidated.

There is a rigorous and well developed theory of fluids that relates the forces between particles (molecules) to statistical distributions of relative particle positions⁸. In our statistical-mechanical analysis we have used the pair distribution function, $g(r)$, which is a measure of the frequency of finding a second particle at a point a distance r away from a given first one. Previous studies of electron micrographs to determine interactions between membrane proteins have been limited to analyses of $g(r)$ ^{9–11}. As $g(r)$ does not in practice uniquely determine the pair force, these studies have necessarily been qualitative and indirect.

To overcome this difficulty, we have in addition used a triplet distribution function, $\rho(s, \theta; r)$, which measures the frequency of encountering a particle at polar coordinates (s, θ) with respect to a given pair of particles separated by r . (See the insert in Fig. 1 for the coordinate system.)

The relation between the pair and triplet functions and the effective force between pairs of particles, $f(r)$, is given by the Born–Green–Yvon (BGY) equation⁸:

$$kT \frac{d[\ln(g(r))]}{dr} = f(r) + \int_0^\infty \int_0^{2\pi} f(s) \cos(\theta) \rho(s, \theta; r) s d\theta ds \quad (1)$$

The left-hand side of the BGY equation may be seen to represent the statistical mean force, $F(r)$, acting on a particle when there is a second particle a distance r away by relating $g(r)$ to a potential, $W(r)$, according to the Boltzmann distribution, $g(r) \equiv \exp(-W(r)/kT)$; then

$$F(r) \equiv -\frac{d(W(r))}{dr} = kT \frac{d[\ln(g(r))]}{dr} \quad (2)$$

The BGY equation states that this total force arises from two sources: the direct force between the two particles, $f(r)$, and the forces from all other particles (the integral). The integrand weights the component along r of the force exerted on the first particle from these other particles, $f(s) \cos(\theta)$, by their average

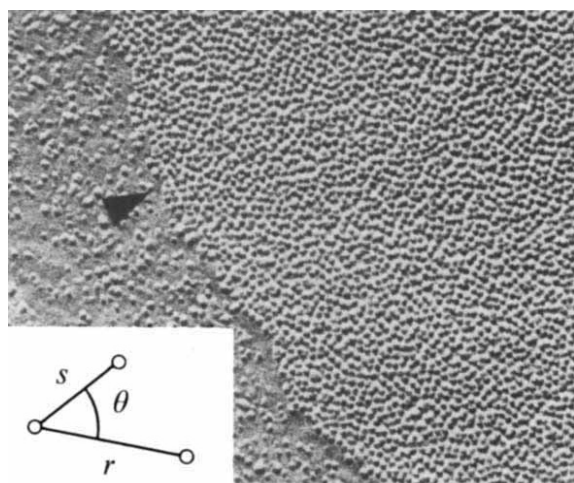


Fig. 1 Freeze-fracture electron micrograph of part of a gap junction from mouse liver, in the open conducting state. This micrograph was made as part of a study by Raviola *et al.*⁴. The gap-junction proteins are seen to be aggregated into a plaque (arrow), at a density of 9,330 particles per μm^2 and an average separation of ~ 10 nm. Particles of similar size in the neighbouring membrane are much more disperse. The inset shows the polar coordinate system used in the analysis of triplet molecular distributions.

number at (s, θ) , $\rho(s, \theta; r)$. The integral extends over the whole membrane, but contributions to it are small for s larger than a few molecular diameters.

The integral over θ can be performed as part of the data acquisition; this reduces the term to a function of r and s that is then tabulated over bins of non-zero width (the integral over s is then approximated as a Riemann sum). The BGY equation reduces to a system of linear equations with the tabulated force as its unique solution. Tabulating the many triplet configurations is a formidable computational task, but it can be made manageable by using suitable algorithms (J.B., J.R.A. and J.C.O., in preparation). To test our method, we performed a Monte Carlo simulation of a simple two-dimensional fluid and recovered the correct forces (attractions as well as repulsions) from samples of the molecular positions (J.B., J.R.A. and J.C.O., in preparation). For the gap junction, the calculated force contains the influences of unobserved degrees of freedom (for example, lipid positions and dyad orientations) and multi-body interactions among dyads in an averaged manner. To reflect this fact, we refer to it as an effective pair force.

Figure 2 shows $g(r)$ calculated for the dyads in the plaque in Fig. 1. Its functional shape is typical of simple fluids. The calculated force between dyads, given in Fig. 3, is entirely repulsive (that is, positive); its range is ~ 12 nm, well beyond the distance at which dyads are in direct contact, which we estimate at no more than 7 nm, based on electron microscopy¹². Several contributions to this force are conceivable, including the effects of intervening lipids. However, volume exclusion at 5–6 nm, together with electrostatic repulsion due to the order of 10 elementary charges per dyad, provides the simplest and best quantified accounting of the observed force; it is also consistent with the suggestion⁷ that the tighter aggregation and crystallization observed when the dyads bind cations and close their pores is due to a reduction of electrostatic repulsion.

The fact that our analysis leads to a simple, physically interpretable force is in itself significant. If the distribution of dyad positions were the result of cytoskeletal anchoring, the calculated force would probably be highly unphysical. Our results thus provide additional evidence that gap junctions represent the equilibrium state of dyads and reflect the forces that act between them. At the same time, our result rules out effective attractions between individual dyads (such as the proposed

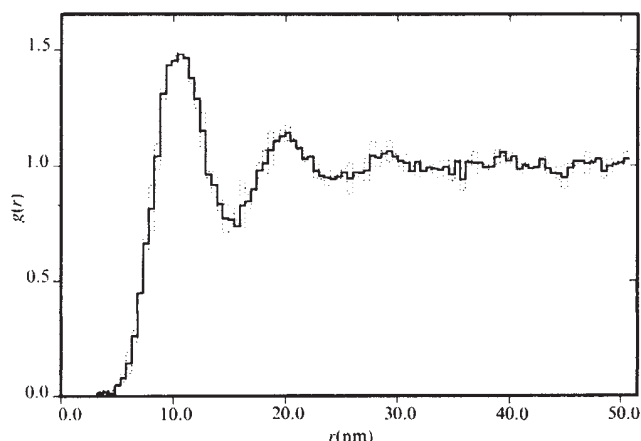


Fig. 2 Pair distribution function, $g(r)$, for dyads inside the plaque in Fig. 1. The uncertainties in digitizing the positions of the centres of the dyads lie in the range 0.5–1.0 nm. The error envelope (dotted line) represents the standard deviations obtained by performing the analysis separately on two parts of the plaque, containing a total of 4,319 dyads. Information contained in $g(r)$ includes the effective diameter of the dyads (the range where $g(r)$ is close to 0), the predominant first neighbour separation (the position of the first peak), and the radial decay of order in successive shells of neighbours about each dyad (the damped oscillatory behaviour of $g(r)$).

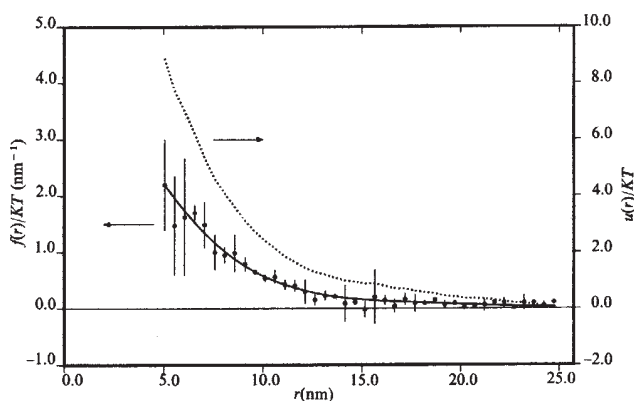


Fig. 3 Effective pair force, $f(r)$ (●), between dyads in the plaque in Fig. 1, computed using the BGY equation. The force was divided by the thermal Boltzmann energy, kT , where $T \sim 310$ K. The error bars for the force were obtained as in Fig. 2. In the range 25–50 nm (not shown) the force is small; the extremes are -0.21 and $+0.17$ nm^{-1} , with error bars ~ 0.1 nm^{-1} . Considering the statistical fluctuations in the data and the accuracy of the numerical methods, there is little evidence that the true force differs significantly from zero in the extended range. The dotted line shows the effective pair potential, $u(r)$, obtained by integrating the force over r ; this is the pair energy required to compress two dyads from a reference separation of 25 nm in the plaque.

specific binding between gap-junction proteins)² that could have explained why dyads are aggregated in plaques. One can, however, imagine several ways in which dyads could aggregate without pair attractions acting inside the plaque. Such mechanisms might arise from the bending elasticity of membranes¹ or from attractions between proteins due to perturbations of nearby lipids^{13–15}.

The mechanism that we find most satisfactory is illustrated schematically in Fig. 4: when a dyad is formed by linking gap-junction proteins in two apposed membranes, surrounding areas of membrane are drawn to within 2 nm of each other¹⁶. In the process, the mutual repulsion of the membranes, partly

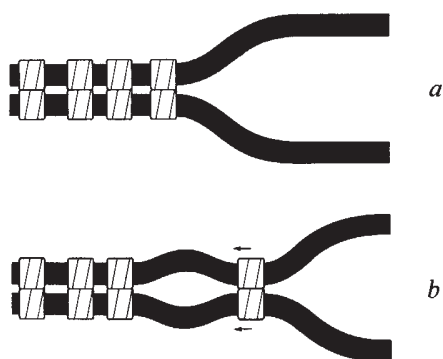


Fig. 4 Hypothetical mechanism of dyad cohesion (schematic). The escape of a dyad from an aggregate ($a \rightarrow b$) increases the area of energetically unfavourable close apposition of the membranes. This provides the driving force for the cohesion. For dyads within the aggregate, however, the force largely vanishes.

electrostatic in nature, must be overcome¹⁷. The total energy expended in overcoming this repulsion is minimized when the area of close apposition is minimized—that is, when the dyads aggregate. We estimate that the electrostatic repulsions alone are sufficiently strong to maintain the observed density of dyads (J.B., J.R.A. and J.C.O., in preparation).

The total area of closely apposed membrane would, however, be largely independent of the positions of the individual dyads in the junction. Accordingly, dyads in the bulk of the junction would not experience this attraction. Isolated pairs of dyads would experience it, as would dyads crossing the boundary of the junction.

This mechanism requires that the two halves of a dyad be bonded quite strongly. The apposed halves of gap junctions are, in fact, difficult to separate¹⁸.

Our model can also explain certain aspects of the formation and disassembly of gap junctions: the formation of conducting dyads precedes the appearance of aggregates of intramembrane particles in Novikoff hepatoma cells that have been dispersed and then allowed to reaggregate¹⁹. In the same system, disperse assemblies of intramembrane particles appear to develop into aggregated ones¹⁸. After dissociation of the cells, gap junctions in which the second membrane is still present (albeit torn loose from its cell) remain intact for some time, whereas intramembrane particle assemblies apparently disperse where dyads have been split¹⁸.

Based on our knowledge of the effective force with which dyads interact, we have outlined how gap junctions may maintain their structure: dyads that repel each other remain aggregated because of a requirement to minimize the area where membranes approach each other closely. Finally, we note that gap junctions vary considerably among tissues and species^{1,2}. It remains to be seen to what extent the forces that we have observed in a mouse liver gap junction operate elsewhere.

We acknowledge the generous gift of micrographs by E. Raviola, as well as computer access by D. Glaser and R. Henry. This work was supported by grants from the NIH (R.S.G. 2-S07-RR07006) and the American Chemical Society/Petroleum Research Fund (12378-G6). J.B. is a fellow of the German National Fellowship Foundation, and J.R.A. is a NIH predoctoral trainee.

Received 30 December 1983; accepted 3 May 1984.

- Loewenstein, W. R. *Physiol. Rev.* **61**, 829–913 (1981).
- Bennett, M. V. L. & Goodenough, D. A. *Neurosci. Res. Prog. Bull.* **16**, 415 (1978).
- Heuser, J. E. & Reese, T. S. in *The Neurosciences Fourth Study Program* (eds Schmitt, F. O. & Worden, F. G.) (MIT Press, Cambridge, Massachusetts, in the press).
- Raviola, E., Goodenough, D. A. & Raviola, G. *J. Cell Biol.* **87**, 273–279 (1980).
- Meller, K. *Anat. Embryol.* **163**, 321–330 (1981).
- Tadvalkar, G. & Pinto da Silva, P. *J. Cell Biol.* **96**, 1279–1287 (1983).
- Peracchia, C. & Peracchia, L. L. *J. Cell Biol.* **87**, 719–727 (1980).
- Hill, T. L. *Statistical Mechanics* Ch. 6 (McGraw-Hill, New York, 1956).
- Markovics, J., Glass, L. & Maul, G. G. *Expl Cell Res.* **85**, 443–451 (1974).
- Perelson, A. S. *Expl Cell Res.* **112**, 309–321 (1978).

- Pearson, T. L., Chan, S. I., Lewis, B. & Engelman, D. M. *Biophys. J.* **43**, 167–174 (1983).
- Unwin, P. N. T. & Zampighi, G. *Nature* **283**, 545–549 (1980).
- Marcelja, S. *Biochim. biophys. Acta* **455**, 1–7 (1976).
- Owicki, J. C. & McConnell, H. M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4750–4754 (1979).
- Edelman, J. B. thesis, California Inst. Technol., Pasadena (1978).
- Goodenough, D. A. *Cold Spring Harb. Symp. quant. Biol.* **40**, 37–43 (1976).
- Rand, R. P. A. *Rev. Biophys. Bioengng* **10**, 277–314 (1981).
- Preus, D., Johnson, R. & Sheridan, J. J. *ultrastruct. Res.* **77**, 248–262 (1981).
- Johnson, R., Hammer, M., Sheridan, J. & Revel, J. P. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4536–4540 (1974).

Expression of functional GABA, glycine and glutamate receptors in *Xenopus* oocytes injected with rat brain mRNA

K. M. Houamed*, G. Bilbe†, T. G. Smart*, A. Constanti*, D. A. Brown*, E. A. Barnard† & B. M. Richards‡

* MRC Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, UK
† MRC Molecular Neurobiology Research Group, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ, UK
‡ Molecular Genetics Department, Searle Research and Development, Lane End Road, High Wycombe, Bucks HR12 4HL, UK

The use of Gurdon's¹ *Xenopus* oocyte translation system has allowed the production of neurotransmitter receptors in a foreign cell membrane, following the translation of microinjected mRNA isolated from various sources^{2–4}. This very accessible and relatively simple preparation permits the study of the requirements for receptor–ionophore function, assembly and membrane integration. This analysis is presently feasible for the peripheral nicotinic acetylcholine receptor^{2,3}, the chick brain γ -aminobutyrate (GABA) receptor⁴ and the rat brain serotonin receptor⁵. We now report the novel and successful expression of the GABA, glycine, glutamate and related acidic amino acid receptors of mammalian brain, and show that they exhibit pharmacologically separate identities when their mRNAs are processed in the amphibian oocyte.

mRNA was extracted from day 14 Wistar (WA/KIR) rats using a guanidinium thiocyanate method⁶. Poly(A) mRNA was isolated by oligo(dT)–cellulose chromatography and injected into *Xenopus laevis* oocytes (~40 ng per cell) as previously reported⁴. After microinjection of the mRNA, cells were maintained in an incubator at 21 °C for 24 h before recording. They were superfused at room temperature (20–23 °C) with frog Ringer containing (mM): NaCl 118; KCl 1.9; CaCl₂ 2.0; Tris 2.5 buffered to pH 7.4 with 1 M HCl. All drugs (BDH or Sigma) were dissolved and applied in this medium. This study was based on data obtained from 47 cells chosen from 4 injected batches of oocytes. Recording conditions and the two-electrode current clamp were as previously described⁴.

All injected oocytes examined responded to bath-applied GABA and glycine by changing their resting membrane potential and conductance; control, non-injected cells showed no sensitivity to these neutral amino acids. Both GABA (2–640 μ M) and glycine (20 μ M–1.6 mM) evoked a 'smooth' membrane potential depolarization (compare with the oscillatory responses to acetylcholine⁷ obtained in uninjected oocytes, and also serotonin⁵ and, in this work, glutamate in mRNA-injected oocytes) which was coupled with an increase in membrane conductance (Fig. 1b). These responses to GABA and glycine were quite reproducible, enabling dose–conductance curves to be constructed (Fig. 1a); GABA was invariably more potent than glycine (2–20-fold, dependent on individual oocytes). The low dose thresholds suggest a minimal influence of uptake mechanisms on the responses, although individual GABA

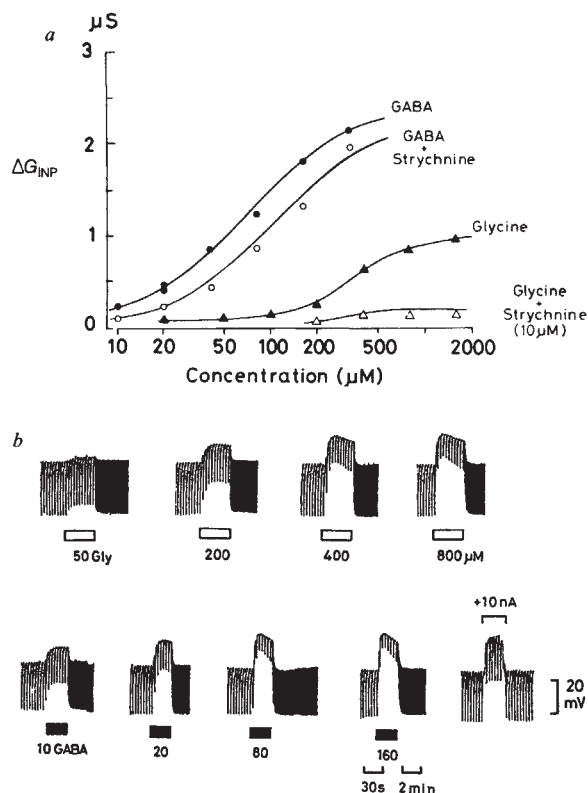


Fig. 1 *a*, GABA and glycine dose-conductance curves in the presence and absence of $10 \mu\text{M}$ strychnine. Data were obtained from a single oocyte by applying control doses of GABA ($\bullet$) and glycine ($\blacktriangle$) in the absence of strychnine and then repeating these in the presence of $10 \mu\text{M}$ strychnine ($\circ$, $\triangle$, respectively). Note the small lateral shift in the GABA curve and the marked 'non-competitive' depression of the glycine curve in antagonist solution. The amino acid evoked input-conductance change (ΔG_{inp}) was uncorrected for membrane rectification. *b*, Membrane conductance changes evoked by bath-applied GABA (lower traces) and glycine (upper traces) in a single oocyte. Downward deflections are hyperpolarizing electrotonic potentials recorded in response to d.c. current injection (20 nA , 1 s duration, 0.25 Hz). Upward deflections of the baseline indicate membrane depolarizations induced by GABA or glycine applied during periods indicated by the bars. Resting potential, -70 mV . GABA produces a greater maximal conductance change than glycine. Desensitization is more apparent in the GABA traces (compare $80 \mu\text{M}$ GABA with $200 \mu\text{M}$ Gly). The evoked conductance changes were largely attributable to the amino acids and not solely due to membrane rectification as shown by the end response in the lower trace, where d.c. current depolarized the cell to match an equivalent GABA response ($160 \mu\text{M}$ GABA) but exhibited a much smaller conductance change.

responses always exhibited a rapid fade or desensitization, as frequently seen in rat central nervous system (CNS) neurones (a feature not observed with responses from chick brain GABA receptors expressed in oocytes⁴). Equivalent glycine responses exhibited less desensitization.

When the membrane potential was progressively depolarized by current injection, previously matched responses (depolarizations) to doses of GABA and glycine decreased in amplitude and eventually reversed at approximately -25 mV (Fig. 2). This reversal potential corresponds very closely with the chloride equilibrium potential in these cells⁷, suggesting that (as expected) chloride ions probably mediate both the GABA and glycine responses.

The apparent conductance changes produced by GABA and glycine were enhanced in the depolarizing direction by membrane rectification. Oocytes injected with mRNA seemed to exhibit a more pronounced rectification than the non-injected controls; perhaps the heterologous mRNA mixture can induce the cell to express a voltage-sensitive rectifying channel in its

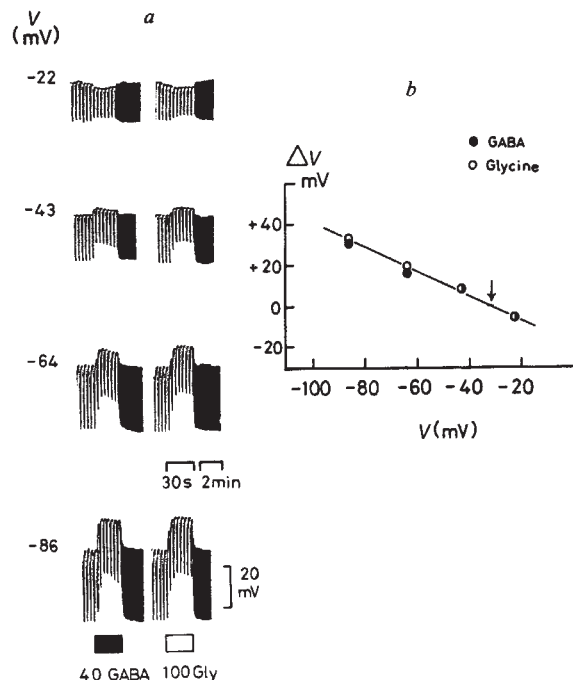


Fig. 2 Measurement of the reversal potential for the GABA- and glycine-induced membrane depolarizations in the same oocyte. *a*, $40 \mu\text{M}$ GABA and $100 \mu\text{M}$ glycine were bath applied and the membrane depolarization was measured at four holding potentials, -22 , -43 , -64 (normal resting potential) and -86 mV . *b*, The change in the induced membrane depolarization (ΔV) is plotted for GABA ($\bullet$) and glycine ($\circ$) against the holding potential (V). Both responses had the same reversal potential, approximately -31 mV . Hyperpolarizing electrotonic potentials were evoked by -25 nA current steps.

membrane (see ref. 8). This rectifier was not blocked by tetraethylammonium (20 mM) or 4-aminopyridine ($10 \mu\text{M}$).

The properties of these neutral amino acid receptors were further investigated using picrotoxinin and strychnine (both antagonists at vertebrate CNS inhibitory synapses^{9,10}), and the GABA potentiators pentobarbitone¹¹ and chlorazepate⁴, a water-soluble benzodiazepine. Strychnine, $1 \mu\text{M}$, inhibited the responses to glycine (Fig. 3*a*) in a 'non-competitive' manner (that is, simply reducing the maximum of the dose-response curve with no lateral shift), with little effect on membrane potential. The glycine responses were slow to recover fully, requiring up to 2 h washing. Using a larger dose ($10 \mu\text{M}$), strychnine blocked not only glycine but also GABA responses (Fig. 1*a*); however, the latter responses were easily recovered on washing. Similar effects of 'high dose' strychnine on GABA responses have been observed previously on CNS neurones¹². Picrotoxinin ($1\text{--}5 \mu\text{M}$) reversibly depressed both GABA and glycine responses (Fig. 3*b*), suggesting that this agent lacks the specificity exhibited by strychnine. (+)Bicuculline ($20 \mu\text{M}$) showed more specificity by reversibly antagonizing GABA in preference to glycine responses. However, GABA responses compared with glycine, were selectively enhanced by $50 \mu\text{M}$ pentobarbitone (Fig. 4*a*), and also by $10 \mu\text{M}$ chlorazepate (Fig. 4*b*).

The preliminary results indicated that these GABA receptors possessed a pharmacological sensitivity that was representative of vertebrate CNS GABA receptors. However, the 'oocyte-expressed' glycine receptors appeared more sensitive to picrotoxinin than their counterparts in native neuronal membranes¹³.

The heterologous mRNA also endowed the oocyte with sensitivity to the acidic amino acids L-glutamate ($10 \mu\text{M}\text{--}1 \text{ mM}$), kainate ($1\text{--}200 \mu\text{M}$), L-aspartate ($100 \mu\text{M}\text{--}2 \text{ mM}$) and quisqualate ($10\text{--}20 \mu\text{M}$) (Fig. 5). Sensitivity to serotonin (1 nM thresh-

hold) and β -alanine (0.3–5 mM) was also observed, but taurine (0.5 mM), *N*-methyl-D-aspartate (100 μ M), L-cysteic acid (100 μ M) and D-aspartate (50 μ M) were inactive. Generally, the excitatory amino acids exhibited the following order of potency on a single oocyte: quisqualate = kainate > glutamate = L-aspartate. The relative potencies were again dependent on the

individual oocyte, quisqualate and kainate being 2–10 times more potent than glutamate and L-aspartate.

Our experiments clearly show the expression of receptor complexes to the two neutral amino acids which are integrally involved with inhibitory neurotransmission in the vertebrate CNS¹⁰. Whereas the role for GABA as an inhibitory neurotransmitter in mammalian brain is reasonably established, comparable evidence for glycine is lacking (compare ref. 14). It is possible that mRNA promotes the expression of glycine receptors even in the possible absence of any discrete glycinergic pathway, for example, non-synaptic receptors. A precedent for this situation does exist in the peripheral nervous system, where most neural tissues contain GABA receptors in the absence of any GABAergic pathway¹⁵.

The GABA receptor from mammalian brain has recently been purified and found to contain several subunits, incorporating the various specific ligand sites for this receptor^{16,17}. Presumably, the mRNA fraction used here contains mRNA molecules coding for each of these subunits; the subunits are subsequently assembled and integrated into the membrane with the formation of their intrinsic ionophore, adopting the correct orientation. The same must be true for the mRNAs coding for the glycine receptor subunits, which have also been isolated as a pure protein¹⁸. It is presently unclear whether these two receptors possess identical chloride ionophores: the evidence, both from patch clamping of cultured neuronal membranes¹⁹ and from our translation results, is compatible with this concept, but not incontrovertible. Our results suggest that the biosynthesized proteins modulating the chloride channel(s) in the receptor complexes are different, because the amino acid-evoked conductance is selectively modified by several different ligands. It was interesting that the receptor for the major excitatory neurotransmitter, glutamate, and several related, putative transmitters can also be assembled in the oocyte.

This mRNA preparation from mammalian brain provides a further example of the faithful post-translational processing at

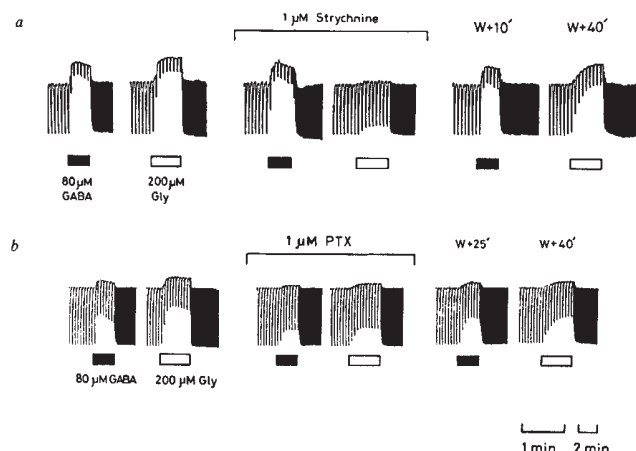


Fig. 3 The effect of the amino acid antagonists strychnine and picrotoxin on the responses to bath-applied GABA (hatched bars) and glycine (open bars) responses obtained from two oocytes. Upward deflections of the trace indicate membrane depolarizations and downward deflections are hyperpolarizing electrotonic potentials (evoked by 15 nA (a) and 24 nA (b)). a, GABA responses are virtually unaltered by 1 μ M strychnine, which preferentially antagonized the glycine response. An almost complete recovery from strychnine was obtained after 40 min washing (W). Single oocyte, resting potential, -47 mV. b, Low-dose picrotoxin (1 μ M) did not exhibit any selectivity for the GABA response as glycine responses were also reduced. Recovery for GABA responses was obtained after 25 min washing in normal Ringer, whereas glycine was still depressed after 40 min. Single oocyte, resting potential, -51 mV.

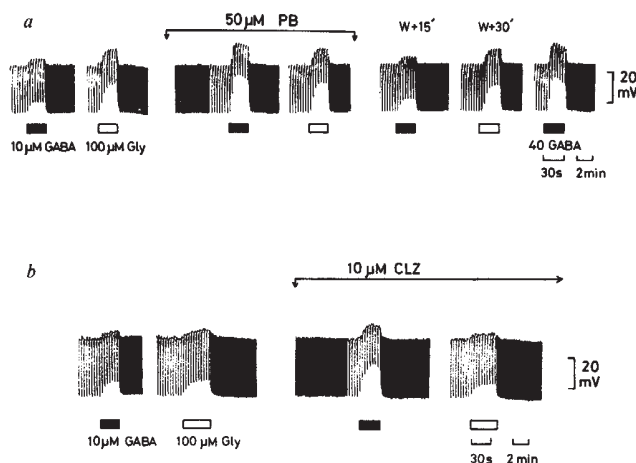


Fig. 4 Enhancement of the GABA-evoked conductance change by 50 μ M pentobarbitone (PB) and chlorazepate (CLZ) in two oocytes. a, 10 μ M GABA and 100 μ M glycine responses (both submaximal) were recorded in control solution, then after 5 and 15 min in 50 μ M PB, respectively, and finally after 15 and 30 min wash in control Ringer. Resting potential, -69 mV. Note that only the GABA response was enhanced, an effect which was easily reversed on washing. The 10 μ M GABA response was enhanced to the level of a 40 μ M GABA response in control solution as shown by the end response in this trace. b, Control responses to 10 μ M GABA and 100 μ M glycine were recorded in normal solution, and then after 7 and 14 min in 10 μ M CLZ, respectively. Resting potential, -62 mV. Only the GABA response was again enhanced but on washing a recovery was not observed as noted previously⁴.

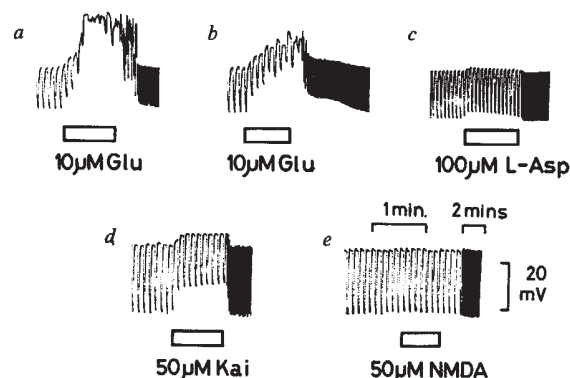


Fig. 5 Membrane conductance changes produced by bath application of excitatory amino acids in *Xenopus* oocytes injected with rat brain mRNA. Records are from four oocytes (a–d). a, Response to 10 μ M L-glutamate (Glu); note the oscillatory nature of the membrane potential and conductance. Recovery was rapid on washing. The low-dose response suggests that the effect of glutamate uptake is minimal. Electrotonic potentials were elicited by -10 nA injected current; resting potential, -81 mV. b, Response to 10 μ M L-glutamate on a different oocyte which is less sensitive to Glu but which still produces membrane potential and conductance oscillations. Current injection, -10 nA; resting potential, -84 mV. c, Response to 100 μ M L-aspartate (L-Asp) on a different oocyte. Note only a small, fading or desensitizing response with no obvious oscillation of the membrane potential or conductance. Current injection, -20 nA; resting potential, -74 mV. d, Response to 50 μ M kainate (Kai). Membrane conductance and potential changes exhibited no fade, desensitization or oscillation and were rapidly reversible on washing. Current injection, -22 nA; resting potential, -62 mV. e, Lack of response to 50 μ M *N*-methyl-D-aspartate (NMDA); same oocyte as in d. D-aspartate (100 μ M) also failed to evoke any response.

which the *X. laevis* oocyte excels. The pharmacological accessibility of this preparation makes a detailed study of amino acid receptors (both neutral and acidic) a reality without the complications presented by an intact CNS.

After submission of this manuscript, Gundersen *et al.*^{20,21} reported the expression of kainate and glycine receptors in the *X. laevis* oocyte, after microinjection with human brain mRNA. Our results with kainate, glycine and rat brain mRNA are largely confirmatory to this study.

G.B. holds a SERC research studentship; K.M.H. received an ORS award. This work was supported by the MRC.

Received 20 February; accepted 16 May 1984.

1. Gurdon, J. B., Lane, C. D., Woodland, H. R. & Marbaix, G. *Nature* **233**, 177–182 (1971).
2. Sumikawa, K., Houghton, M., Emtage, J. S., Richards, B. M. & Barnard, E. A. *Nature* **292**, 862–864 (1981).
3. Barnard, E. A., Miledi, R. & Sumikawa, K. *Proc. R. Soc. B* **215**, 241–246 (1982).
4. Smart, T. G., Constanti, A., Bilbe, G., Brown, D. A. & Barnard, E. A. *Neurosci. Lett.* **40**, 55–59 (1983).
5. Gundersen, C. B., Miledi, R. & Parker, I. *Proc. R. Soc. B* **219**, 103–109 (1983).
6. Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294–5299 (1979).
7. Kusano, K., Miledi, R. & Stinnakre, J. *J. Physiol., Lond.* **328**, 143–170 (1982).
8. Gundersen, C. B., Miledi, R. & Parker, I. *Proc. R. Soc. B* **220**, 131–140 (1983).
9. Nistri, A. & Constanti, A. *Prog. Neurobiol.* **13**, 117–236 (1979).
10. Curtis, D. R. & Johnston, G. A. R. *Ergebn. Physiol.* **69**, 97–188 (1974).
11. Barker, J. L. & McBurney, R. N. *Proc. R. Soc. B* **206**, 319–327 (1979).
12. Curtis, D. R., Duggan, A. W. & Johnston, G. A. R. *Expl Brain Res.* **12**, 547–565 (1971).
13. Simmonds, M. A. *Br. J. Pharmac.* **79**, 799–806 (1983).
14. Levi *et al.* *Brain Res.* **236**, 121–131 (1982).
15. Brown, D. A., Higgins, A. J., Marsh, S. & Smart, T. G. in *Amino Acid Neurotransmitters* (eds DeFeudis, F. V. & Mandel, P.) 321–325 (Raven, New York, 1981).
16. Sigel, E., Stephenson, F. A., Mamalaki, C. & Barnard, E. A. *J. biol. Chem.* **258**, 6965–6971 (1983).
17. Sigel, E., Mamalaki, C. & Barnard, E. A. *FEBS Lett.* **147**, 45–48 (1982).
18. Pfeiffer, F., Graham, D. & Betz, H. *J. biol. Chem.* **257**, 9389–9393 (1982).
19. Hamill, O. P., Bormann, J. & Sakmann, B. *Nature* **305**, 805–808 (1983).
20. Gundersen, C. B., Miledi, R. & Parker, I. *Nature* **308**, 421–424 (1984).
21. Gundersen, C. B., Miledi, R. & Parker, I. *Proc. R. Soc. B* **221**, 235–244 (1984).

High-affinity binding sites for abscisic acid on the plasmalemma of *Vicia faba* guard cells

Claudia Hornberg & Elmar W. Weiler

Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität, Postfach 102148, D-4630 Bochum, FRG

Stomata have a key role in the regulation of gas exchange and water economy in higher plants^{1,2}. During water stress, they close in response to abscisic acid (ABA), a hormonal stimulus thought to be released or produced *de novo* in the mesophyll of leaves^{3,4}, and probably in the guard cells of the stomata themselves⁵. By a mechanism still unknown, ABA affects the ionic balance of guard cells, resulting in a loss of K⁺, their major osmotically active constituent^{1,6}. The structural requirements for a compound to be physiologically active in this system are stringent⁷ and suggest a highly specific interaction of the hormone with its primary target site(s). Apart from two preliminary studies^{8,9}, ABA binding has not been investigated in plant material. Using guard cell protoplasts^{10,11}, we have now obtained evidence for the occurrence of high-affinity guard cell-specific ABA-binding proteins which are located at the plasmalemma of these cells with high density. In all characteristics examined, they bear the properties of ABA receptors.

ABA contains an α,β -unsaturated ketone moiety which can be photoactivated by irradiation with light of ~ 330 nm (ref. 12). This provides a means of irreversibly labelling potential ABA receptors in the living cell with the unmodified hormone. We have cross-linked ABA to its putative binding sites by irradiating guard cell protoplasts of *Vicia faba* L. after incubation with low levels of the physiologically active ³H-cis(+)ABA. The biologically inactive (–)enantiomer served as a control. Irradiations of bovine serum albumin solutions in the presence of ABA proved that both enantiomers were cross-linked to exactly the

same extent (Fig. 1a, insert). However, photoinduced cross-linking of ABA with guard cell protoplasts showed a pronounced specificity for the physiologically active enantiomer (Fig. 1a). Mesophyll cell protoplasts of the same plant were labelled to a much lesser extent, but also exhibited discrimination of the ABA enantiomers (Fig. 1a). Both the kinetics of ABA binding (Fig. 1a) and the effect of pH on hormone binding (Fig. 1b) were compatible with the kinetics and pH dependence of ABA-induced stomatal closure in a *V. faba* epidermal strip bioassay (Fig. 1b), suggesting a physiological relevance of the binding sites. The sites were readily saturated with cis(+)ABA (Fig. 1c) and the binding of labelled hormone was reversible with cis(+)ABA but not with the physiologically inactive enantiomer (Fig. 1d). Approximately 75% of the total cross-linked cis(+)ABA was associated with high-affinity sites (Fig. 1d). From the data (Fig. 1c, d), we estimated an apparent K_D for the intact guard cell protoplast system for cis(+)ABA of ~ 3 –4 nM, which corresponds well with the physiological concentration range of the hormone (~ 5 nM gave half-maximum rates of stomatal closure in *V. faba* epidermal strip bioassays). The total number of the high-affinity sites was estimated to be 3.2 amol per protoplast (19.5×10^5 sites).

Mild tryptic treatment of guard cell protoplasts before incubation with ABA completely eliminated the ABA-binding sites (Table 1), but left the protoplasts intact, as shown by their ability to accumulate Neutral red and to exclude phenosafranin. The results indicate that the binding sites are proteins that are located at the plasmalemma of guard cells, with the ABA-binding area facing the apoplasmic space. This conclusion is consistent with the observed pH effects on ABA binding: at pH 8.5, when binding is still readily observable, practically all the hormone ($pK = 4.7$)¹³ is present as the anion (ABA[–]), for which plasma membranes have been shown to be impermeable¹³. Assuming an even distribution, the plasmalemma density of the ABA-binding sites was estimated as $2,200 \mu\text{m}^{-2}$ (protoplast diameter $\sim 17 \mu\text{m}$).

Treatment with SDS partly solubilized the photoaffinity-labelled binding proteins. On SDS-polyacrylamide gel electrophoresis (PAGE), three proteins, cross-linked specifically with cis(+)ABA, with molecular weights (M_r) 20,200, 19,300 and 14,300, were detected and designated sites A, B and C. The labelling pattern of proteins from mesophyll protoplasts was distinctly different from that of the guard cell proteins (Fig. 2), proving that the ABA-binding sites are specifically or at least much more strongly expressed in the hormone-responsive guard

Table 1 Trypsin treatment of intact guard cell protoplasts before incubation with ABA abolishes ABA binding

ABA enantiomer	Radioactivity of photoaffinity-labelled protein (d.p.m. per μg protein)	
	Trypsin-treated	Control
cis(+)ABA		
Expt 1	43 $\pm$ 33	22,854 $\pm$ 4,098
2	18 $\pm$ 22	24,295 $\pm$ 1,810
3	68 $\pm$ 64	23,109 $\pm$ 1,363
4	10 $\pm$ 10	25,564 $\pm$ 1,443
cis(–)ABA		
Expt 1	98 $\pm$ 76	5,361 $\pm$ 123
2	168 $\pm$ 193	5,064 $\pm$ 1,050
3	127 $\pm$ 54	5,347 $\pm$ 238
4	35 $\pm$ 24	5,377 $\pm$ 162

Protoplasts (23×10^4 cells ml^{-1}) prepared as described in Fig. 1 legend were incubated with $5 \mu\text{g ml}^{-1}$ (expts 1, 2) or $9 \mu\text{g ml}^{-1}$ (expts 3, 4) trypsin for 10 min at 22 °C. After the addition of $30 \mu\text{g ml}^{-1}$ trypsin inhibitor, cells were incubated in 4.2 nM ³H-cis(+)ABA and ³H-cis(–)ABA, respectively. Photoinduced cross-linking and processing were as described for Fig. 1. Controls were treated identically except that the trypsin inhibitor was added before trypsin. The data are average $\pm$ s.d. of triplicate sets of each experiment. The integrity of the cells was checked after the treatments by their ability to accumulate Neutral red and exclude phenosafranin¹⁷ (>95% of viable cells).

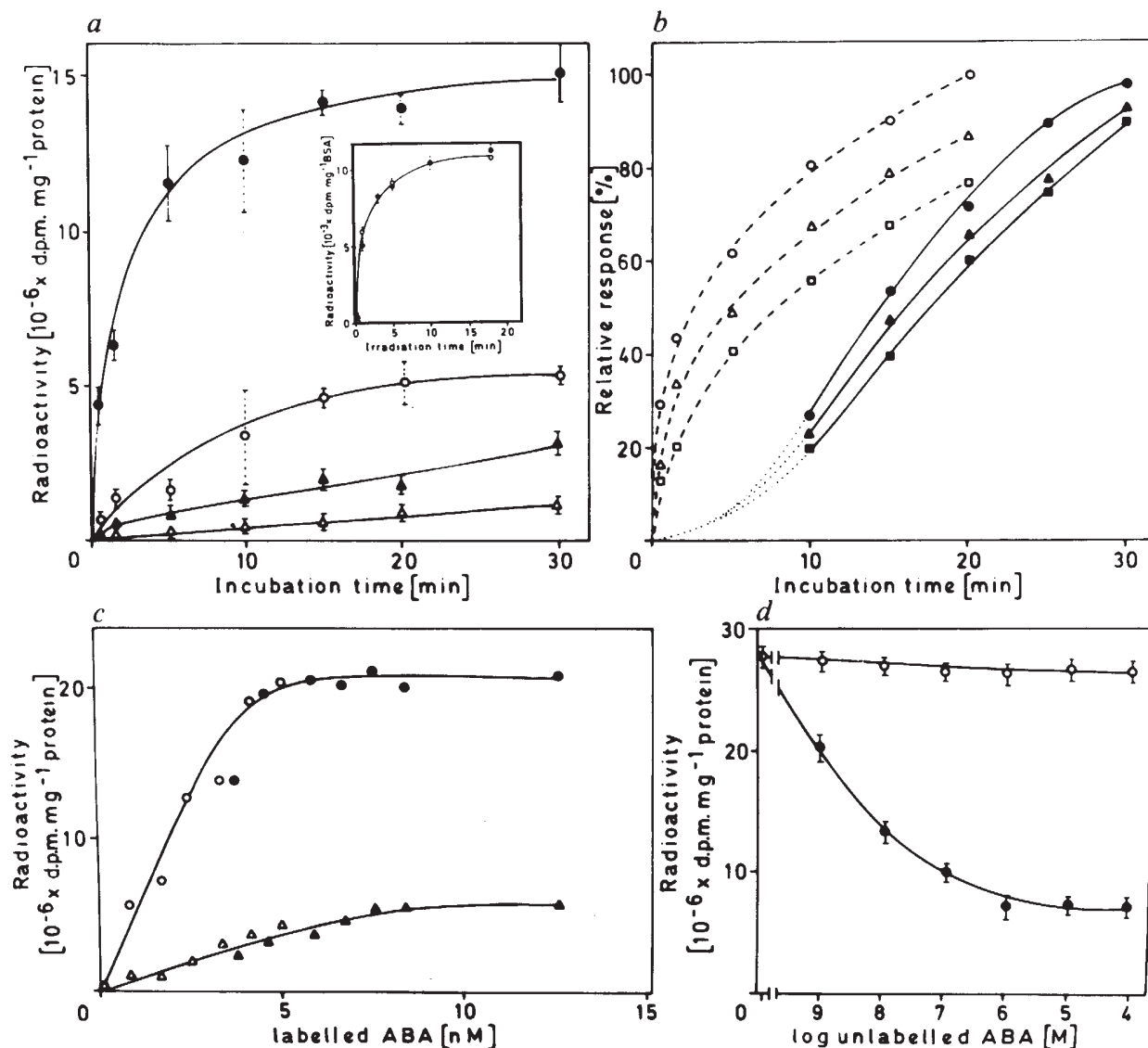


Fig. 1 Characterization of ABA binding to guard cell protoplasts of *V. faba* L. **a**, Kinetics of binding of ^3H -cis-(+)-ABA (39 Ci mmol $^{-1}$, 4.2 nM final concentration) to guard cell protoplasts at pH 6.5 (●). Controls: ^3H -cis-(−)-ABA (39 Ci mmol $^{-1}$, 4.2 nM) binding to guard cell protoplasts (○), ^3H -cis-(+)-ABA (▲) and ^3H -cis-(−)-ABA (△) binding to mesophyll protoplasts of the same plant. Average $\pm$ s.d. of nine (solid bars) or three (dashed bars) experiments. The insert shows photoinduced cross-linking at pH 6.5 of ^3H -cis-(+)-ABA (●) and ^3H -cis-(−)-ABA (○), 4.2 nM, with bovine serum albumin (BSA, 2.5 mg ml $^{-1}$, incubation time 30 min) as a function of irradiation time. Average $\pm$ s.d. of three experiments. **b**, Kinetics of binding of ^3H -cis-(+)-ABA (4.2 nM) to guard cell protoplasts of *V. faba* as affected by pH (dashed lines) compared with the effect of pH on ABA-induced (50 nM) stomatal closure in epidermal strips in epidermal strips (solid lines). ○, ●, pH 4; △, ▲, pH 6.5; □, ■, pH 8.5. To facilitate comparison, the data were plotted on a relative scale assigning the 100% value respectively, to ABA cross-linked after incubation for 20 min at pH 4 and to complete stomatal closure observed after 30 min of incubation of epidermal strips in ABA solution at pH 4. **c**, Saturation analysis of guard cell protoplast binding sites with ^3H -cis-(+)-ABA (expt 1, ○; expt 2, ●) compared with the effect of ^3H -cis-(−)-ABA (expt 1, △; expt 2, ▲), both incubated for 30 min at pH 6.5. **d**, Displacement of ^3H -cis-(+)-ABA (4.2 nM) by unlabelled, immunoaffinity-purified ^{18}cis (+)-ABA (●) and ^{18}cis (−)-ABA (○) at pH 6.5. Average $\pm$ s.d. of nine experiments. Total number of high-affinity sites = 274 pmol per mg protein, equivalent to 1.9×10^6 sites per cell.

Methods: Guard cell protoplasts were prepared from epidermal strips of 3-week-old *V. faba* L. cv. 'Hangdown grünkern' as described previously^{5,10} but using 1% of pretreated¹⁹ cellulysin. Only preparations with >95% intact protoplasts (see Table 1) and with <3% mesophyll contamination were subsequently used. A suspension of 23×10^4 protoplasts ml $^{-1}$ in 0.6 M mannitol, 1 mM CaCl $_2$, 10 mM Na-ascorbate, 10 mM Na-phosphate, pH 6.5 or as indicated (adjusted with HCl or NaOH and checked at the beginning and end of the incubation period) was incubated on ice in the dark with 4.2 nM ^3H -cis-(+)-ABA or 4.2 nM ^3H -cis-(−)-ABA. The enantiomers, prepared by immunoaffinity chromatography¹⁸, had the same specific activity (39 Ci mmol $^{-1}$) and enantiomeric purity (>98%). After the incubation, the cells were rapidly washed twice in the above medium without hormone and re-pelleted by centrifugation at 120g for 5 min. They were then reconstituted to the original cell density and immediately irradiated at ≥ 320 nm (Schott WG 320 long pass filter, 1 mm thick) using a HBO 500 W/2 high-pressure mercury lamp (Osram)¹². Irradiation was always carried out for 10 min with constant cooling (10 °C). The cross-linked radioactivity was determined in the trichloroacetic acid-precipitable protein²⁰ which was recovered on a glass fibre filter (Schleicher and Schüll no. 6). In non-irradiated controls, <0.1% of the radioactivity precipitated from irradiated samples was retained by the filters. Protein determinations were made in supernatants of lysed (H $_2$ O) protoplasts with the Biorad assay and BSA as reference protein; protein content per guard cell protoplast was 11.7 pg BSA equivalents. Bioassay: Freshly prepared epidermal strips (average stomatal aperture¹ 18 μ m) were spread in 10 mM CaCl $_2$, 10 mM Na-ascorbate, pH 6.5, on microscope slides and covered with a coverslip. The buffered (pH 6.5) solution of the test compound (0.1 μ M; control: buffer) was then introduced by suction and was frequently replaced to ensure constant incubation conditions. At the appropriate times, stomatal apertures were determined by microscopic observation¹. Measurements from 30 stomata were averaged, each experiment being repeated three times. Mesophyll protoplasts were prepared by incubating leaf samples, with the lower epidermis removed, for 2 h at 30 °C in 0.4 M mannitol with 4% cellulysin, 10 mM CaCl $_2$ and 50 mM Na-ascorbate, pH 6.5. The crude protoplast fraction was pelleted by centrifugation at 120g (1 min) and washed twice with the above solution, omitting the cellulysin. The washed pellets were resuspended in 4 ml of 0.5 M sucrose with 5% Ficoll and overlaid with 4 ml 0.5 M sucrose, then 3 ml 0.5 M mannitol (all with 1 mM CaCl $_2$, 10 mM Na-ascorbate, pH 6.5). After centrifugation, the protoplast fraction collected from the mannitol-sucrose interphase was washed in 0.5 M mannitol, 10 mM CaCl $_2$, 50 mM Na-ascorbate, adjusted to 23×10^4 cells ml $^{-1}$, then treated exactly as the guard cell preparations.

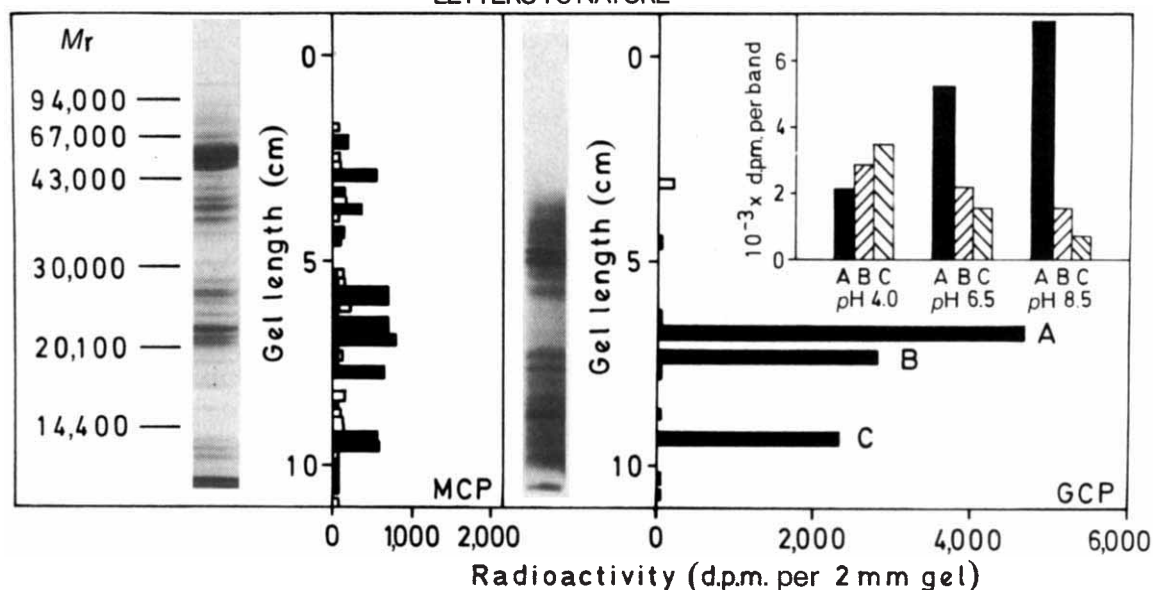


Fig. 2 SDS-PAGE of photoaffinity-labelled mesophyll (MCP, left) and guard cell (GCP, right) protoplast proteins after incubation for 30 min in 4.2 nM ^3H -*cis*(+)-ABA (solid bars) or ^3H -*cis*(-)-ABA (open bars). Protoplast preparation, incubation (pH 6.5) and irradiation as in Fig. 1. Inset: Radioactivity recovered from bands A, B and C of SDS-PAGE of guard cell protoplast proteins after incubation with 4.2 nM ^3H -*cis*(+)-ABA at different pHs. All data are based on 10 μg total protein.

Methods: Irradiated guard cell or mesophyll protoplasts were pelleted and lysed in 60 μl buffer containing 3% SDS, 10% glycerol and 5% β -mercaptoethanol in 0.05 M Tris-HCl buffer at pH 6.8. Precipitated mannitol and cell debris were removed by centrifugation and the supernatants were subjected to SDS-PAGE²¹ using 12.5% gels, 1 mm thick, and a constant current of 10 mA for 12–14 h. Protein was stained with Coomassie blue then the gels were sliced into 1-mm segments. Two segments were combined, solubilized with H_2O_2 (ref. 22) and their radioactivity determined.

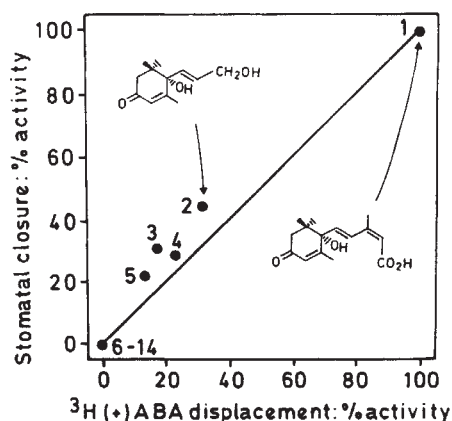


Fig. 3 Correlation between the effect of 0.1 μM solutions (pH 6.5) of *cis*(+)-ABA (1) and ABA-related compounds (2–14) on the induction of stomatal closure in *V. faba* (ordinate) and on competition with ^3H -*cis*(+)-ABA for its guard cell binding sites (abscissa). For the latter test, ABA analogues were incubated with the labelled *cis*(+)-ABA. Relative activities in the bioassay were estimated by comparing the final stomatal aperture after equilibration of the system (30 min) with the test compounds (0% closure, buffer control; 100% closure, obtained after 30 min in the presence of *cis*(+)-ABA). For the binding assay, relative activities are expressed as the relative amount of tracer displaced from the high-affinity binding sites (0% displacement, buffer control; 100% displacement, residual (nonspecific) binding in the presence of 0.1 μM unlabelled *cis*(+)-ABA). 1: *cis*(+)-ABA; 2: vomifolol; 3: α -ionylidene acetic acid; 4: ABA-4'-tyrosylhydrazone; 5: 3-desmethyl ABA; 6: *cis*(-)-ABA; 7: *trans*-ABA; 8: *cis*(+)-ABA methyl ester; 9: *cis*-ABA glucosyl ester; 10: all-*trans* farnesol; 11: dihydrophaseic acid; 12: *cis*-xanthoxin; 13: 2-*cis*-epoxy- β -ionylidene acetic acid; 14: indole-3-acetic acid.

cells. Site A was labelled preferentially when guard cell protoplasts had been incubated with the hormone at alkaline pH whereas sites B and C were most strongly labelled after an acidic incubation (Fig. 2, insert). Control experiments established that sites B and C were not degradation products of site A originating during SDS-PAGE. Even though solubilization and separation

of the labelled ABA-binding proteins in non-denaturing conditions has not yet been achieved, the available evidence is compatible with the existence of three distinct ABA-binding sites, one of which (site A) binds ABA⁻, the other two probably binding the protonated hormone. In such a system, total ABA binding (and consequently hormone recognition) would become largely independent of apoplasmic pH. In support of this conclusion, ABA induces stomatal closure of *V. faba* (Fig. 1b), as well as of other species¹⁴, at acidic as well as basic pH.

Further evidence suggesting that the ABA-binding proteins described here represent the receptor(s) mediating the hormone-induced stomatal closure comes from the close correlation between the physiological activity and the ability to displace ^3H (+)-ABA from its high-affinity binding sites, for a range of ABA analogues (Fig. 3). Thus, vomifolol¹⁵, α -ionylidene acetic acid, 3-desmethyl-ABA and the 4'-tyrosylhydrazone of ABA were active, whereas (-)-ABA, *trans*-ABA, *cis*(+)-ABA methyl ester and glucose ester, the putative ABA precursor xanthoxin, and the ABA catabolite dihydrophaseic acid, were inactive in both test systems (see Fig. 3).

The extensive study of hormone binding in plants¹⁶ has so far—largely because of the lack of an appropriate experimental system—provided little conclusive information about the primary sites and the mechanism(s) of plant hormone action. This is the first demonstration that specific, high-affinity hormone-binding proteins that are likely to be involved in the process of hormone recognition and action occur at the plasmalemma of hormone-responsive cells in higher plants. It is hoped that further study of the system described here will promote our understanding of the mechanisms underlying plant hormone action.

We thank Professors O. Pongs and K. Schäfer for an introduction to the techniques of photoaffinity-labelling and for permission to use equipment during several stages of this work. We also thank Professor Yamashita for the gift of several ABA analogues. Funding by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie, Frankfurt, to E.W.W. is gratefully acknowledged.

Received 13 February; accepted 26 April 1984.

1. Raschke, K. in *Encyclopaedia of Plant Physiology* New Ser., Vol. 7 (eds Haupt, W. & Feinleib, M. E.) 381–441 (Springer, Berlin, 1979).

2. Zeiger, E. A. *Rev. Pl. Physiol.* **34**, 441-475 (1983).
3. Mansfield, T. A., Wellburn, A. R. & Moreira, T. J. S. *Phil. Trans. R. Soc. B* **284**, 471-482 (1978).
4. Pierce, M. & Raschke, K. *Planta* **148**, 174-182 (1980).
5. Weiler, E. W., Schnabl, H. & Hornberg, C. *Planta* **154**, 24-28 (1982).
6. Outlaw, W. H. Jr *Physiologia Pl.* **59**, 301-311 (1983).
7. Cummins, W. R., Kende, H. & Raschke, K. *Planta* **99**, 347-351 (1971).
8. Hocking, T. J., Clapham, J. & Catell, K. J. *Planta* **138**, 303-304 (1978).
9. Curvetto, N., Delmastro, S. & Brevedan, R. *Pl. Physiol.* **69**, Suppl., 40 (1982).
10. Schnabl, H., Bornmann, C. H. & Ziegler, H. *Planta* **143**, 33-39 (1978).
11. Weyers, J. D. B. et al. *Physiologia Pl.* **58**, 331-339 (1983).
12. Gronemeyer, H. & Pongs, O. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2108-2112 (1980).
13. Kaiser, W. & Hartung, W. *Pl. Physiol.* **68**, 202-206 (1981).
14. Hartung, W. *Pl. Cell Envir.* **6**, 427-428 (1983).
15. Pousset, J.-L. & Poisson, J. *Tetrahedron Lett.*, 1173-1174 (1969).
16. Kende, H. & Gerdner, G. A. *Rev. Pl. Physiol.* **27**, 267-320 (1976).
17. Widholm, J. *Stain Technol.* **47**, 189-194 (1972).
18. Mertens, R., Stünning, M. & Weiler, E. W. *Naturwissenschaften* **69**, 595-596 (1982).
19. Boller, T. & Kende, H. *Pl. Physiol.* **63**, 1123-1132 (1979).
20. Merrill, C. R., Switzer, R. C. & Van Keuren, M. L. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4335-4339 (1979).
21. O'Farrell, P. H. *J. biol. Chem.* **250**, 4007-4021 (1975).
22. Oettmeier, W. *FEBS Lett.* **118**, 267-270 (1980).

Human T-cell leukaemia virus is not lysed by human serum

Hiroo Hoshino*, Hiroko Tanaka*, Masanao Miwa* & Hidechika Okada†

* National Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo 104, Japan

† Department of Microbiology, Fukuoka University School of Medicine, Nanakuma, Johanna-ku, Fukuoka 814-01, Japan

Retroviruses isolated from avian, feline, murine and simian sources have been found to be inactivated and lysed by normal human serum¹⁻³. There is much evidence that complement is activated directly by retroviruses in the absence of antibody⁴⁻⁷. Thus, human complement is thought to function as a natural defence mechanism against horizontal infection by retroviruses. Recently, a novel retrovirus, human T-cell leukaemia virus (HTLV), has been shown to be associated with adult T-cell leukaemia/lymphoma (ATL)⁸⁻¹⁶. A large number of healthy adults in south-west Japan^{12,17}, the West Indies^{18,19} and Africa^{20,21} carry antibodies against HTLV and these seropositive individuals are considered to be carriers of HTLV²²⁻²⁴. Thus, horizontal spread of HTLV occurs frequently among humans. We set out to determine whether HTLV reacts with human complement, and report here that, unlike other animal retroviruses, HTLV is not lysed by normal human serum—this might explain the infectivity and persistence of HTLV in humans.

HTLV was purified from the culture fluids of MT-2 (ref. 13), ATL-2M and CaL-2 cells. ATL-2M cells were derived from

peripheral blood lymphocytes of an ATL patient¹⁶ and CaL-2 cells were established by co-cultivation of peripheral blood lymphocytes from a cat with lethally irradiated MT-2 cells (unpublished data). Moloney murine leukaemia virus (M-MuLV) and bovine leukaemia virus (BLV) were prepared from the culture fluids of YAC-1 (ref. 25) and FLK cells²⁶, respectively. Normal human sera were obtained from five healthy laboratory workers; these sera had no antibody activity against MT-2, YAC-1 or FLK cells as judged by indirect immunofluorescence.

The reactivities of HTLV, M-MuLV and BLV with human complement were assayed by the method of Welsh et al.^{1,4-6} (Table 1). Each viral suspension was mixed with an equal volume of fresh human serum, heated human serum, Nonidet P-40 (NP40) or TNE buffer (see Table 1 for description of the buffer). Treatment with NP40 resulted in the release of reverse transcriptase from HTLV produced by MT-2, ATL-2M and CaL-2 cells, from M-MuLV produced by YAC-1 cells and from BLV produced by FLK cells. However, neither fresh nor heated human serum caused any release of reverse transcriptase from HTLV, whereas fresh human serum was effective in releasing reverse transcriptase from M-MuLV as reported elsewhere^{1,2,4-6} (Table 1). Fresh human serum also released reverse transcriptase even from BLV, which is related to HTLV only distantly²⁷. All of five normal human sera lysed M-MuLV and BLV but not HTLV. We also tested the effects of other animal sera on HTLV lysis. Reverse transcriptase assays indicated that HTLV produced by MT-2 cells was not lysed by serum from cat, guinea pig, mouse, rabbit or rat.

Further, we tested whether HTLV was resistant to antibody-mediated virolysis. Serum from several ATL patients or a healthy HTLV antibody carrier was used as a source of antibody and fresh human serum as a source of complement. We found that HTLV from MT-2 and ATL-2M cells was also resistant to antibody-mediated virolysis. Serum obtained from normal adults or ATL patients had no inhibitory effect on the reverse transcriptase activity of HTLV disrupted by NP40 (data not shown). We cannot explain why fresh human serum failed to lyse HTLV in the presence of anti-HTLV antibody.

Recently, we have demonstrated that the amplification reaction of complement is inhibited on homologous cell membranes^{28,29}. Therefore, it seemed likely that HTLV might be resistant to human complement because the virus incorporated human membrane constituents that inhibited amplification of human complement. To exclude this possibility, we tested the susceptibility to human complement of HTLV produced by the feline T-cell line, CaL-2. Table 1 shows that there was no release

Table 1 Effects of human serum on the release of reverse transcriptase from HTLV, M-MuLV and BLV

Virus	Cell line (species)	Treatment			
		Fresh	Heated	NP40	TNE
HTLV	MT-2 (human)	150 ± 10	150 ± 10	31,150 ± 1,250	110 ± 30
	ATL-2M (human)	190 ± 10	180 ± 10	5,030 ± 170	140 ± 10
	CaL-2 (cat)	540 ± 40	510 ± 110	1,940 ± 70	450 ± 40
	YAC-1 (mouse)	31,510 ± 870	480 ± 30	62,740 ± 1,500	380 ± 70
M-MuLV	FLK (sheep)	10,860 ± 1,850	740 ± 140	59,270 ± 280	1,670 ± 40

HTLV was prepared from MT-2, ATL-2M and CaL-2 cells, M-MuLV from YAC-1 cells and BLV from FLK cells. ATL-2M cells were maintained as described elsewhere¹⁶. FLK cells were maintained in Eagle's minimum essential medium containing 10% fetal calf serum (FCS) and the other cells in RPMI 1640 medium containing 10-20% FCS. The culture fluids of these cells were centrifuged for 2 h at 87,000g. The precipitates were suspended in TNE buffer (20 mM Tris-HCl pH 7.5, 100 mM NaCl, 1 mM EDTA). HTLV produced by MT-2 cells was further purified by continuous sucrose density gradient (20-60%) centrifugation for 20 h at 87,000g. Fractions at densities of ~1.15 g l⁻¹ were pooled and used. HTLV prepared without sucrose density gradient centrifugation gave similar results. Reverse transcriptase was assayed in duplicate essentially as described by Welsh et al.^{1,4-6}. The viral suspension (10 µl) was mixed with 10 µl of fresh or heated (56 °C, 30 min) human serum and incubated for 30 min at 37 °C. NP40 (0.2%) in TNE buffer and TNE buffer alone were used as controls. The reaction mixture (90 µl) containing 40 mM Tris-HCl pH 7.8, 4 mM dithiothreitol, 45 mM KCl, 15 µM ³H-thymidine 5'-triphosphate, 100 µM deoxyadenosine 5'-triphosphate, 100 µM deoxycytidine 5'-triphosphate, 100 µM deoxyguanosine 5'-triphosphate, 9 µg poly(rA), 1.8 µg oligo(dT)₁₂₋₁₈ and either 10 mM MgCl₂ or 0.25 mM MnCl₂ was then added to the viral suspension mixture. For the assay of reverse transcriptase, MgCl₂ was used with HTLV and BLV and MnCl₂ with M-MuLV. After incubation for 1 h at 37 °C, 50-µl samples of each assay mixture were spotted onto DE81 filter papers. The filters were washed with 5% Na₂HPO₄, water and ethanol, then the radioactivity bound to the filters was counted in a liquid scintillation counter. Values are the mean (c.p.m.) ± s.d.

of reverse transcriptase from HTLV produced by CaL-2 cells on treatment with fresh human serum. Thus, HTLV from cat cells also was not lysed by human serum.

To observe any virolysis not detected by the above method, we also examined the sedimentation rate of HTLV after treatment with human serum. MT-2 and YAC-1 cells were maintained in RPMI 1640 medium containing ^3H -uridine. After incubation for 20 h, the culture fluid was collected, centrifuged for 2 h at 87,000g, and the precipitate suspended in TNE buffer. The viral suspension was mixed with an equal volume of either fresh human serum or heated human serum and incubated at 37 °C for 30 min. Then the viral suspension was layered onto a continuous sucrose gradient (20–60%) and centrifuged at 87,000g for 20 h at 4 °C. After fractionation, we found that HTLV treated with fresh human serum formed a radioactive peak at a density of $\sim 1.15 \text{ g l}^{-1}$, reported to be the buoyant density of HTLV produced by MT-2 cells¹⁴. In contrast, M-MuLV treated with fresh human serum did not show any peak, while M-MuLV treated with heated human serum formed a peak at a density

of $\sim 1.17 \text{ g l}^{-1}$, as reported previously¹ (data not shown). This finding indicates that M-MuLV RNA, but not HTLV RNA, was released from virus particles by treatment with fresh human serum.

Thus, to our knowledge, HTLV represents the first retrovirus that is resistant to human complement. It remains to be determined whether HTLV also retains infectivity on incubation with human serum. So far, however, titration of cell-free HTLV was not been achieved, although Clapham *et al.* succeeded in cell-free analysis of HTLV infection using pseudotypes of vesicular stomatitis virus bearing HTLV envelope antigens³⁰. The finding that HTLV is not lysed by human complement may explain why so many adults are seropositive for HTLV and are probably HTLV carriers.

This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Culture and Science and from the Ministry of Health and Welfare of Japan and by a Grant-in-Aid from the Society for Promotion of Cancer Research, Tokyo.

Received 23 February; accepted 24 May 1984.

1. Welsh, R. M. Jr, Cooper, N. R., Jensen, F. C. & Oldstone, M. B. A. *Nature* **257**, 612–614 (1975).
2. Cooper, N. R. *Compreh. Virol.* **15**, 123–170 (1979).
3. Weiss, R. in *Molecular Biology of Tumor Viruses, RNA Tumor Viruses* (eds Weiss, R., Teich, N., Varmus, H. & Coffin, J.) 1205–1281 (Cold Spring Harbor Laboratory, New York, 1982).
4. Welsh, R. M. Jr, Cooper, N. R., Jensen, F. C. & Oldstone, M. B. A. *Virology* **74**, 432–440 (1976).
5. Bartholomew, R. M., Esser, A. F. & Muller-Eberhard, H. J. *J. exp. Med.* **147**, 844–853 (1978).
6. Cooper, N. R., Jensen, F. C., Welsh, R. M. Jr & Oldstone, M. B. A. *J. exp. Med.* **144**, 970–984 (1976).
7. Sherwin, S. A., Benveniste, R. E. & Todaro, G. J. *Int. J. Cancer* **21**, 6–11 (1978).
8. Poiesz, B. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 7415–7419 (1980).
9. Poiesz, B. J., Ruscetti, F. W., Reitz, M. S., Kalyanaraman, V. S. & Gallo, R. C. *Nature* **294**, 268–271 (1981).
10. Blattner, W. A., Takatsuki, K. & Gallo, R. C. *J. Am. med. Ass.* **26**, 1074–1080 (1983).
11. Yoshida, M. *Gann* **74**, 777–789 (1983).

12. Hinuma, Y. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 6476–6480 (1981).
13. Miyoshi, I. *et al. Nature* **294**, 770–771 (1981).
14. Yoshida, M., Miyoshi, I. & Hinuma, Y. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2031–2035 (1982).
15. Popovic, M. *et al. Science* **219**, 851–859 (1983).
16. Hoshino, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 6061–6065 (1983).
17. Hinuma, Y. *et al. Int. J. Cancer* **29**, 631–635 (1982).
18. Blattner, W. A. *et al. Int. J. Cancer* **30**, 257–264 (1982).
19. Blattner, W. A. *et al. Lancet* **ii**, 61–64 (1983).
20. Hunsmann, G., Schneider, J., Schmitt, J. & Yamamoto, N. *Int. J. Cancer* **32**, 329–332 (1983).
21. Flemming, A. F. *et al. Lancet* **ii**, 334–335 (1983).
22. Miyoshi, I. *et al. Gann* **73**, 339–340 (1982).
23. Gotoh, Y., Suganuma, K. & Hinuma, Y. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4780–4782 (1982).
24. Sarin, P. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 2370–2374 (1983).
25. Cikes, M., Friberg, S. & Klein, G. *J. natn. Cancer Inst.* **50**, 347–362 (1973).
26. Van der Maaten, M. J. & Miller, J. M. *Bibliotheca haemat.* **43**, 360–362 (1976).
27. Oroszlan, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 1291–1294 (1982).
28. Okada, H., Tanaka, H. & Okada, N. *Eur. J. Immun.* **13**, 340–344 (1983).
29. Okada, H. & Tanaka, H. *Molec. Immun.* **20**, 1233–1236 (1983).
30. Clapham, P., Nagy, K. & Weiss, R. A. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2886–2889 (1984).

Restricted number of chromosomal regions implicated in aetiology of human cancer and leukaemia

Felix Mitelman

Department of Clinical Genetics, University Hospital, S-221 85 Lund, Sweden

It has been known since the days of Boveri¹ that neoplasia is associated with chromosomal aberration. The introduction, some 10 years ago, of chromosome banding techniques provided the impetus for the description of an immense number of such aberrations, and for the localization to individual chromosome bands of the breaks underlying the aberrations. Hypothetically, the breaks should comprise two essentially different kinds: primary breaks that are actively involved in the malignant development, and secondary breaks, coincidental to this process. In the search for a possible method to identify primary breaks in human cancer, I selected from the catalogue of chromosome aberrations now available² those cases that had one single structural aberration as their sole deviation from normality. I report here that the breakpoints thus specified affect a surprisingly limited number of chromosomal regions, and conclude that these regions contain genes of prime importance to cancer development.

Nonrandom and specific chromosome aberrations have been described in several malignant disorders of animals and man^{3–6}. The aberrations tend to cluster to certain chromosome types and common duplicated and deleted segments have been mapped^{7–10}. These findings are compatible with the hypothesis that chromosome aberrations have a fundamental role in the initiation of the malignant process. Different models have been proposed to explain the action of such chromosome aberrations by gene amplification and/or gene activation^{9–14}. However, most

Table 1 Fifty-four chromosome changes found in at least two neoplasms with a single structural chromosomal abnormality

t(1; 6)(q23; p22)	del(7)(q11)
t(1; 10)(q21; q23)	del(7)(q22)
t(1; 11)(q21; q23)	t(8; 14)(q24; q32)
t(1; 12)(q21; p13)	t(8; 21)(q22; q22)
t(1; 17)(p36; q21)	t(8; 22)(q24; q11)
dup(1)(q21q31)	t(9; 11)(p21; q23)
dup(1)(q23)	t(9; 11)(p22; q24)
i(1q)	t(9; 11)(p21; q13)
t(2; 8)(p12; q24)	t(9; 22)(q34; q11)
t(3; 8)(p21; q12)	t(9; 22)(q34; q12)
t(3; 8)(p25; q21)	del(9)(q13)
t(3; 11)(q21; p15)	t(11; 14)(q13; q32)
t(3; 12)(p21; q24)	t(11; 17)(q23; q23)
dup(3)(q25)	t(11; 21)(q25; q11)
del(3)(p25)	t(11; 22)(q25; q11)
del(3)(q21q26)	del(11)(q14)
t(4; 11)(q21; q23)	del(11)(q23)
t(4; 11)(q13; q22)	del(13)(q13)
del(5)(q13)	del(13)(q21)
del(5)(q14)	t(14; 18)(q32; q21)
del(5)(q15)	t(15; 17)(q21; q12)
del(5)(q12q31)	t(15; 17)(q22; q12)
del(5)(q13q31)	t(16; 16)(p13; q22)
del(5)(q13q33)	i(17q)
del(5)(q14q34)	del(17)(q11)
t(6; 9)(p23; q34)	del(20)(q11)
del(6)(q23q25)	del(22)(q11)

neoplasms described to date are not characterized by consistent or specific chromosome changes. In fact, most human tumours show seemingly unique, usually complex, structural and/or numerical karyotypic changes and all of the 329 bands of the standard human karyotype¹⁵ have been affected in different types of cancer and leukaemia². The important question is whether all, most, or perhaps only a few of the confusing variety

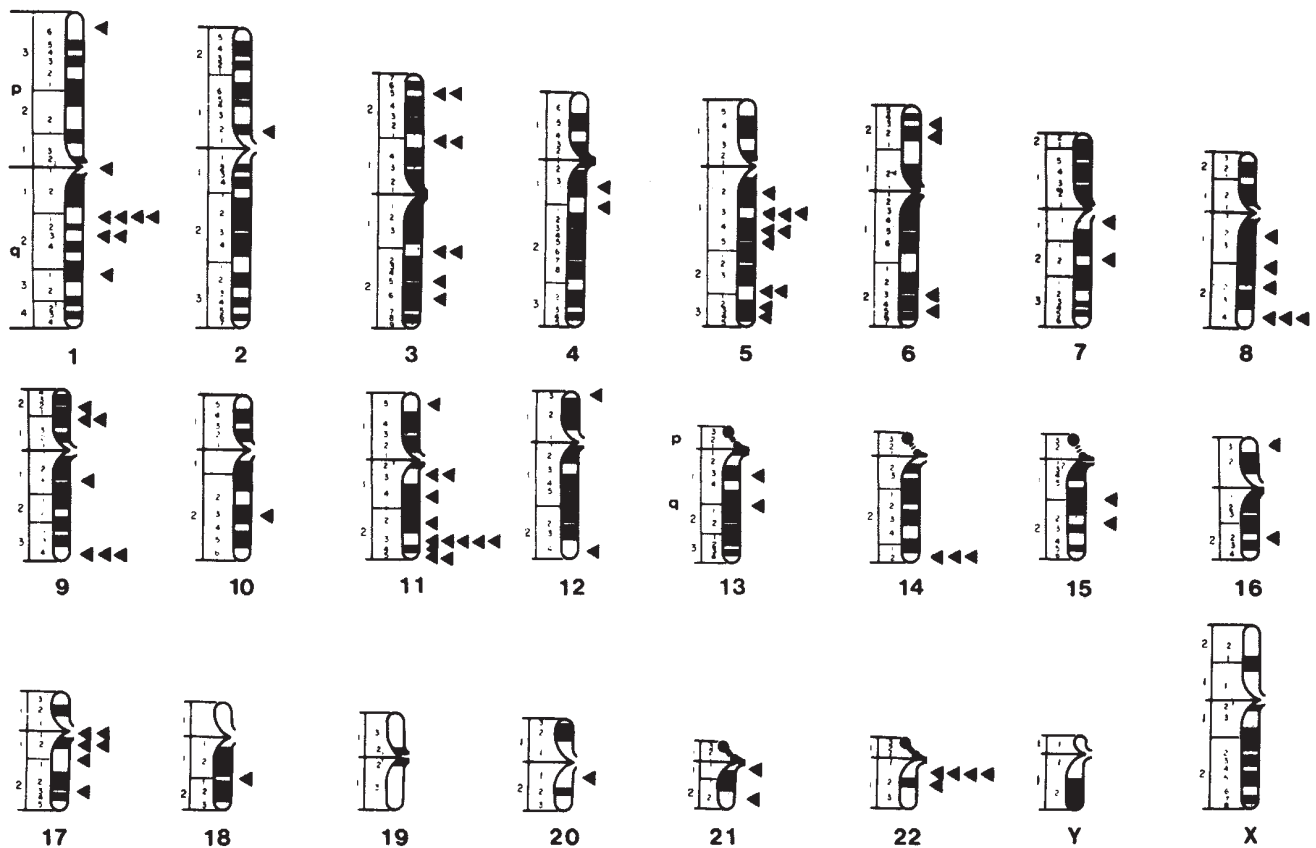


Fig. 1 Distribution over the banded human karyotype of 90 breakpoints underlying structural aberrations in neoplasms with one single deviation from normality (see Table 1). The breakpoints are restricted to 61 bands and the overall pattern suggests that the breakpoints cluster preferentially to specific chromosome regions.

of karyotypic changes present in most tumours are of decisive importance in tumour development. The possibility cannot be excluded that the majority of changes represent random secondary events—the consequences rather than causes of the malignant state.

If chromosome change, either involving gene amplification or gene activation, triggers malignant development of a normal cell, the study of neoplasms having one single aberration should help to identify chromosomal segments of critical significance. In every neoplasm with a single abnormality, this particular aberration apparently may have been sufficient to produce the malignant state. On the basis of this idea, all neoplasms with a single structural chromosome abnormality were ascertained from the registry of chromosome aberrations in human cancer cells², and their breakpoints recorded. Thus, all cases with a reciprocal translocation, inversion, deletion or duplication as the sole abnormality were considered. Among the 3,844 cases reviewed, 226 such different types of aberrations were found. Some of these abnormalities, such as the specific chromosome changes closely related with particular neoplasms, for example,

t(9;22)(q34;q11) in chronic myeloid leukaemia, t(15;17)(q22;q12) in acute promyelocytic leukaemia, t(8;21)(q22;q22) in acute myeloid leukaemia, t(4;11)(q21;q23) in acute lymphocytic leukaemia, del(5)(q13q31) in refractory anaemia and t(8;14)(q24;q32) in Burkitt's lymphoma, were the sole abnormality in a large number of cases; others were found only in a single case. In an attempt to minimize the effects of possible mistakes in karyotype identification and interpretation which may blur any pattern, only those abnormalities were selected that were identical in at least two different tumours. A total of 54 such aberrations were ascertained; these are listed in Table 1. It should be noted that each of these aberrations represented the only deviation from the normal diploid karyotype in at least two neoplasms. The breakpoints of these aberrations are presented in Fig. 1. Variant translocations in chronic myeloid leukaemia have not been included as recent results¹⁶ indicate that, just as in the standard cases with t(9;22), the band 9q34 is involved in these cases even when this is not detectable cytogenetically.

Figure 1 illustrates that a limited number of bands apparently

Table 2 Correspondence between breakpoints of Fig. 1 and those of aberrations in 172 neoplasms with a simple structural aberration as the sole abnormality

Translocations, inversions					Deletions, duplications				
No. of neoplasms	Exact agreement in both chromosomes	Exact agreement in one chromosome, involvement of adjacent band in the other	Exact agreement in one chromosome, no agreement in the other	Adjacent band affected in one chromosome, no agreement in the other	No agreement	No. of neoplasms	Exact agreement in one or both bands involved	Adjacent band affected	No agreement
	38	19	57	8	5		26	11	8

are affected in different types of cancer and leukaemia having only a single abnormality. Thus, even though the 90 breakpoints identified in the 54 different types of aberrations are distributed over all chromosome types except chromosome 19 and the sex chromosomes, the breakpoints are restricted to a total of 61 bands. The following comparison between the breakpoints in Fig. 1 and those of structural aberrations in all other malignant disorders so far reported with either another single abnormality or complex karyotypic changes suggests that these particular breakpoints may represent primary initial changes in carcinogenesis.

First, the 61 different breakpoints identified were compared with those of all 172 neoplasms ascertained to have a unique simple structural aberration as the sole abnormality. Table 2 shows that there was an exact agreement for one or both breakpoints in 114 of 127 translocations and inversions and in 26 of 45 deletions and duplications. Note that in 19 additional cases (8 translocations/inversions and 11 deletions/duplications) the adjacent band was involved—the usually poor quality of tumour chromosomes often makes it extremely difficult to discriminate between adjacent bands and thus these 19 cases, too, may well represent the same breakpoint. If this is the case, 159 of the 172 cases (92.4%) share one of the breakpoints identified in Fig. 1. Adding the 54 aberrations present in two or more cases, that is, the aberrations that form the basis of Fig. 1, the total agreement as regards breakpoint involvement is 213 of 226, that is, 94.2%.

Second, the breakpoints of Fig. 1 were compared with those of all complex karyotypic aberrations of the 3,844 cases presented in the catalogue of chromosome aberrations in cancer²; in this comparison I considered all cases in which each structural aberration had been exactly identified. An exact agreement with at least one of the 61 breakpoints identified in this study was found for 86.7% of cases and an additional 9.4% showed involvement of at least one adjacent band. Thus, 96.1% of the neoplasms studied displayed breakpoints involving the chromosomal segments under consideration.

The data presented here demonstrate that only a limited number of breakpoints are involved regularly in chromosomal aberrations of human neoplasia. Furthermore, the overall pattern suggests that the breakpoints cluster to relatively few chromosomal regions. This notion is strengthened when the considerable sources of error affecting the assignment of breaks to single bands in chromosomes of neoplastic cells are taken into consideration. I conclude that genes of importance for the transformation of a normal cell to a cancerous one are located in a restricted number of chromosomal regions. By using modern techniques of molecular genetics it should be possible to identify and define the nature, expression and interaction of the genes involved.

This study was supported by grants from the Swedish Cancer Society and the John and Augusta Persson Foundation. I thank Professor Albert Levan and Professor Georg Klein for helpful comments on the manuscript.

Received 21 February; accepted 4 May 1984.

1. Boveri, T. *Zur Frage der Entstehung maligner Tumoren* (Fischer, Jena, 1914).
2. Mitelman, F. *Cytogenet. Cell Genet.* **36**, 1–515 (1983).
3. Sandberg, A. A. *The Chromosomes in Human Cancer and Leukemia* (Elsevier, New York, 1980).
4. Spira, J. in *Chromosomes and Cancer* (eds Rowley, J. D. & Ulmann, J.) 85–97 (Academic, New York, 1983).
5. de la Chapelle, A. & Berger, R. *Cytogenet. Cell Genet.* **37**, 274–311 (1983).
6. Mitelman, F. in *Chromosomes and Cancer* (eds Rowley, J. D. & Ulmann, J.) 61–84 (Academic, New York, 1983).
7. Levan, G., Ahlström, U. & Mitelman, F. *Hereditas* **77**, 263–280 (1974).
8. Rowley, J. D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5729–5733 (1977).
9. Mitelman, F. & Levan, G. *Hereditas* **89**, 207–232 (1978).
10. Mitelman, F. & Levan, G. *Hereditas* **95**, 79–139 (1981).
11. Cairns, J. *Nature* **289**, 353–357 (1981).
12. Klein, G. *Nature* **294**, 313–318 (1981).
13. Pall, M. L. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2465–2468 (1981).
14. Rowley, J. D. *Nature* **301**, 290–291 (1983).
15. ISCN *Cytogenet. Cell Genet.* **21**, 309–404 (1978).
16. Bartram, C. R. et al. *Nature* **306**, 277–280 (1983).

A conserved sequence at c-myc oncogene chromosomal translocation breakpoints in plasmacytomas

Steven P. Piccoli*, Perry G. Caimi* & Michael D. Cole*

Department of Biochemistry, St Louis University School of Medicine, 1402 South Grand Blvd, St Louis, Missouri 63104, USA

Many recent studies have shown that chromosomal translocation breakpoints frequently occur near cellular proto-oncogenes (reviewed in ref. 1). In both mouse plasmacytomas and Burkitt lymphomas, the c-myc oncogene becomes joined to an immunoglobulin heavy-chain gene in a head-to-head configuration^{2–7}. Within c-myc, the breaks frequently occur near the first exon–intron boundary⁸, while within the immunoglobulin gene the breaks usually involve sequences directing heavy-chain switching^{2,3,9,10}. It has been assumed that the translocations represent abortive immunoglobulin switching events which have activated the c-myc gene for a role in tumour formation. However, sequence analysis of the c-myc gene does not reveal any apparent similarity to the immunoglobulin switch signals^{11–13}. With these results in mind, we have determined the precise breakpoints within c-myc for two plasmacytoma lines in order to search for any common features that may shed some light on the mechanism of chromosomal translocation. We report here that the tetranucleotide sequence GAGG occurs close to the breakpoint in five out of six translocations, and so may be a sequence recognized by either the enzymes that catalyse immunoglobulin heavy-chain switching, or some other DNA-cleaving activity.

We have previously described the cloning and characterization of the two chromosomal translocation breakpoints involving the c-myc oncogene from BALB/c plasmacytomas MOPC

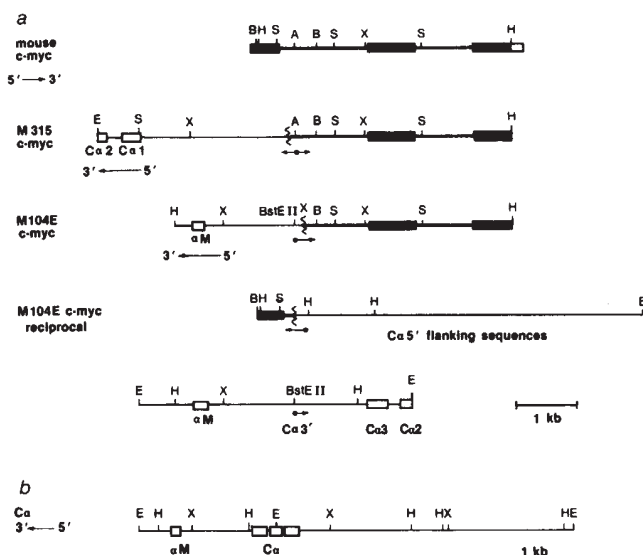


Fig. 1 Restriction enzyme maps of the c-myc and C_{α} clones used in this study. The c-myc genes from MOPC 315 and MOPC 104E have been described previously². The reciprocal of the MOPC 104E translocation was isolated from a Charon 4 library of tumour DNA using the 1.0-kb *HindIII* fragment from the 5' flanking sequences of C_{α} . The *BamHI*–*EcoRI* fragment shown was subcloned into pBR322. The 4.5-kb *EcoRI* fragment containing the 3' flanking sequences of C_{α} and the α membrane domain has been described previously¹⁶. The regions that were sequenced^{24,25} to map the chromosomal translocation breakpoints are indicated by arrows. Restriction enzyme sites are: A, *AvaI*; B, *BamHI*; E, *EcoRI*; H, *HindIII*; S, *SstI*; X, *XbaI*. Only the *AvaI* and *BstEII* sites used for sequence determination are shown.

* Present address: Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA.

315 and MOPC 104E². Restriction maps of these clones are shown in Fig. 1. Figure 2 shows the sequences of the MOPC 315 translocation and the MOPC 104E reciprocal translocation breakpoints. In MOPC 315, there is a distinct point at which the *c-myc* sequences end and non-*c-myc* sequences begin. The sequences to the left of the breakpoint appear to derive from a section of the C_α switch region that has not been sequenced previously as there are numerous repeat sequences near the break analogous to those identified by Davis *et al.*¹⁴. However, there are no switch repeats immediately adjacent to the breakpoint.

The sequences of the two clones from MOPC 104E represent a very different configuration. A large portion of the C_α gene and a segment of *c-myc* are absent from the two halves of the translocation, indicating that this is not a precise reciprocal rearrangement. The displaced first *c-myc* exon is joined to S_α sequences, demonstrating that this translocation originally involved a C_α switch region that was in a germ-line configura-

tion. The sequence of this clone allowed the position of the primary translocation event in MOPC 104E to be determined within *c-myc*.

Characterization of the translocated *c-myc* coding regions in MOPC 104E indicated that the gene was not linked to the 5' side of the C_α gene as in other tumour lines, but instead to sequences derived from the 3' side of the C_α gene. Yet, from analysis of the reciprocal translocation presented above, one would have expected to find the *c-myc* coding region linked to the C_α coding region as in other plasmacytomas. The most likely explanation for this finding is that the translocated *c-myc* gene in MOPC 104E suffered a second DNA rearrangement in which the C_α coding sequences were deleted during the initial translocation or subsequent tumour passage (Fig. 3). We have analysed the DNA sequence at this breakpoint to determine where the secondary deletion occurred in *c-myc* and whether or not any S_α sequences remained at the junction.

Figure 2b shows the sequence surrounding the translocation

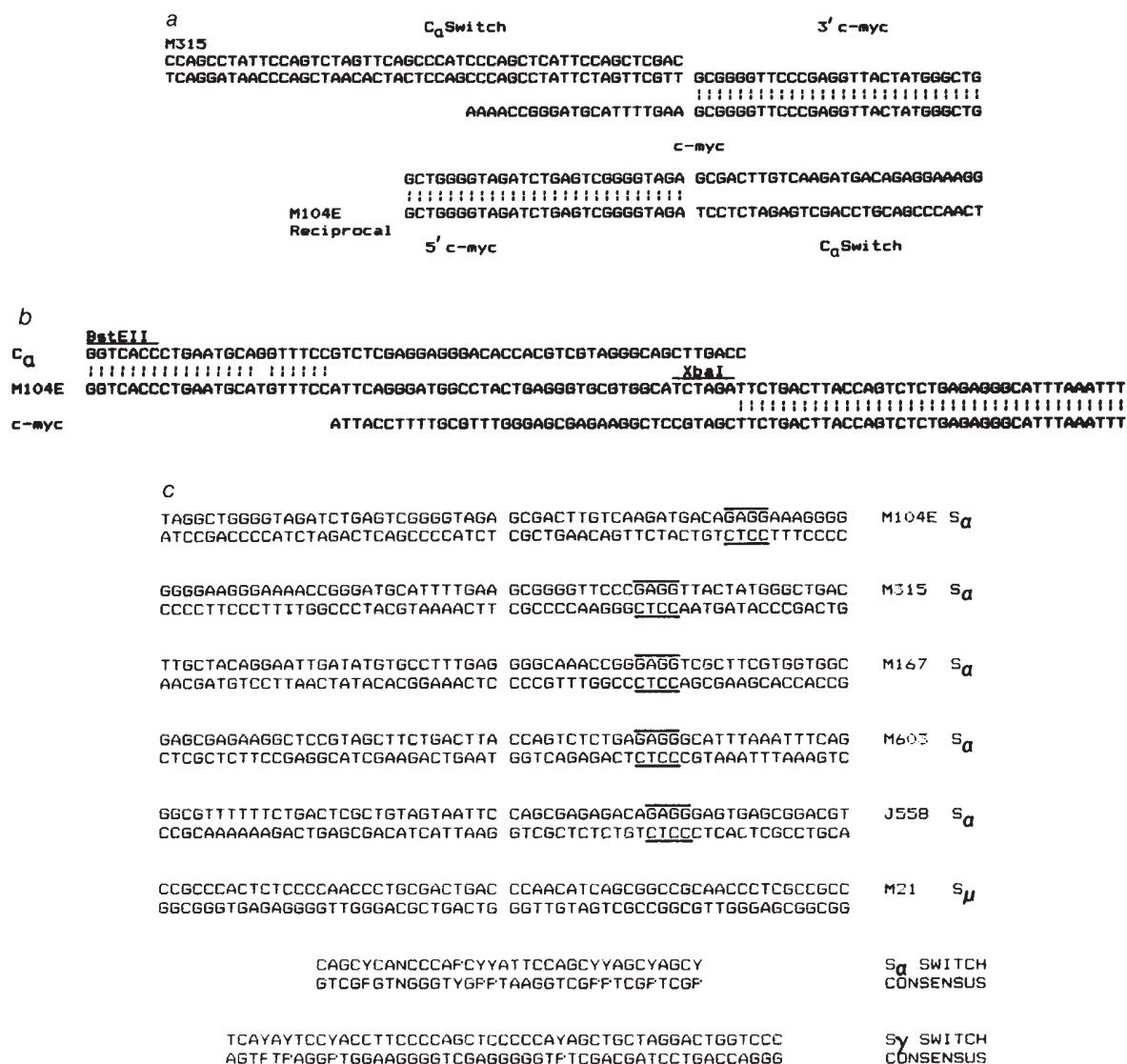


Fig. 2 *a*, Sequences of the MOPC 315 *c-myc* translocation breakpoint and the MOPC 104E reciprocal breakpoints. In MOPC 315, the C_α switch region that is joined to *c-myc* is folded to align the switch-like repeats, which can be compared with the consensus C_α switch sequence shown in *c*. In MOPC 104E, the first *c-myc* exon is joined to the 5' flanking sequence of C_α as shown in Fig. 1. Regions of homology between *c-myc* and the translocation breakpoint are indicated by vertical bars for both clones. *b*, Sequence of the MOPC 104E breakpoint and corresponding regions from the 3' side of the C_α and the *c-myc* intron. The 38 bp separating the two genes is as yet of unknown origin. *c*, *c-myc* sequences at the translocation breakpoints in six plasmacytomas. Each line shows the sequence of *c-myc* surrounding the translocation breakpoint. The sequences to the left of the space are displaced by the immunoglobulin gene, while the sequences to the right of the space remain linked to the *c-myc* coding regions. Both strands of *c-myc* are shown for each tumour so that they can be compared with the immunoglobulin switch repeats. The GAGG sequence that is found 11 or 12 nucleotides from each of the C_α rearrangements is underlined, as is a related sequence in the S_γ repeat.

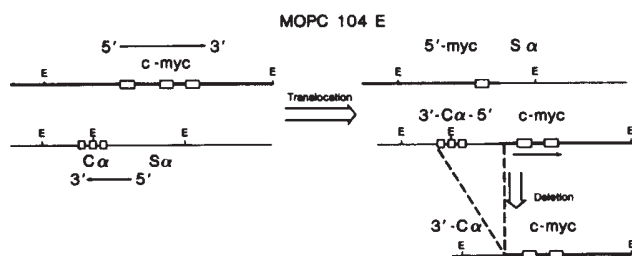


Fig. 3 Diagram of the probable origin of the chromosomal translocation breakpoint in MOPC 104E. The initial rearrangement involved the germ-line C_α gene as shown by the translocation reciprocal in Fig. 1. The α switch and coding regions were subsequently deleted during either the initial rearrangement or tumour passage.

breakpoint in MOPC 104E, together with the relevant portions of *c-myc* and the 3' C_α flanking sequence. The latter was determined from the 4.5-kilobase (kb) *EcoRI* fragment containing the germ-line C_α flanking region¹⁵. The breakpoint within *c-myc* occurs 152 base pairs (bp) closer to the *c-myc* coding region than the reciprocal translocation shown in Fig. 2a (see Fig. 4), indicating that an additional segment of the *c-myc* intron has been deleted together with C_α . On the left side of the sequence, there was a near perfect match to the 3' C_α flanking sequence from a *BstEII* site that was present in both genes. However, between C_α and *c-myc* there were 38 nucleotides that were not identifiable in either gene. These additional sequences do not derive from the published portions of the C_α coding¹⁶ or switch¹⁴ regions but may be retained from other regions of C_α that have not been sequenced, because they have some similarity to the α switch region. Thus, there may have been two DNA breaks involved in the secondary rearrangement of the MOPC 104E gene. More importantly, neither the C_α coding sequences nor the S_α region are required for transcription of the truncated *c-myc* gene in plasmacytomas. Thus, if novel enhancing elements that influence transcription of the *c-myc* gene exist in the immunoglobulin domain, they may be present on the 3' side of the constant-region genes rather than within the coding or switch regions.

As each of the chromosomal translocations described to date appears to be the result of an abortive immunoglobulin switching event, it was important to determine whether there were any common features of the breakpoints within *c-myc* that could be related to the consensus switch sequences. Therefore, we aligned the DNA sequences at the breakpoints in MOPC 315 and MOPC 104E with four sequences taken from previous studies (Fig. 2c). Five of these translocations involve S_α sequences while the sixth involves S_μ . We found a common tetranucleotide sequence (GAGG) 11 or 12 nucleotides from the break for every S_α -*c-myc* breakpoint; MOPC 104E represents somewhat of an exception to this consensus, as the GAGG occurs 19 nucleotides from the breakpoint. However, the position of the breakpoint within *c-myc* for MOPC 104E was determined only from the reciprocal and not from the C_α -*c-myc* coding region breakpoint as in the other cases. In the two translocations for which both parts of *c-myc* have been sequenced, there are 7–11 nucleotides of *c-myc* deleted^{17–19}. Therefore, it is likely that the GAGG in the original MOPC 104E translocation would have been positioned 7–11 bp closer to the breakpoint, which would place it 11 or 12 bp from the breakpoint, in agreement with the other plasmacytomas. The GAGG sequence was not present in the *c-myc* sequence adjacent to the S_μ translocation. Further examination of the sequence surrounding the *c-myc*- S_α breakpoints did not reveal any other conserved sequences on either side of the breakpoint, nor was there any homology with the consensus switch sequences shown in Fig. 2c.

One of the striking features of chromosomal translocations in mouse plasmacytomas is their localization to a 1.4-kb *HindIII*-*BamHI* segment² of the *c-myc* gene which spans the

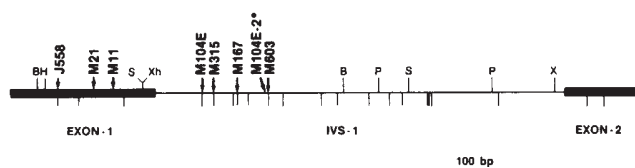


Fig. 4 Distribution of translocation breakpoints in the *c-myc* oncogene. The diagram shows the first *c-myc* intron and the positions of the breakpoints for which the nucleotide sequence is known (upper vertical lines). The lower vertical lines indicate the positions of GAGG sequences in the *c-myc* gene. The position of the secondary deletion for MOPC 104E is indicated as M104E-2. Previously published sequences include J558 (refs 11, 26), M603 (ref. 10), M167 (ref. 10), M21 (refs 17, 18) and M11 (ref. 19).

first exon and part of the first intron. Many breakpoints have been further localized to a region of only 0.5 kb⁸. Figure 4 shows that seven translocation breakpoints are within this exon-intron boundary region. Furthermore, four of the *c-myc*- S_α breaks are localized within a 300-bp segment. This region of chromosome 15 clearly represents a preferred site for translocation events which activate the *c-myc* oncogene.

As yet, the role of the GAGG sequence in chromosomal translocations is unknown. The only counterpart to the *c-myc* GAGG sequence found in consensus switch signals is a GGAG present in all of the γ switch regions as part of more extensive 45–90-bp repeats²⁰ (see Fig. 2c). However, this sequence is on the opposite strand from the GAGG in *c-myc*. Thus, if the GAGG sequence is recognized as having minimal homology with a γ switch, the recombinases may be recognizing a signal on the strand opposite from that which is normally used in productive intrachromosomal immunoglobulin heavy-chain recombination. An alternative possibility for the involvement of GAGG in chromosomal translocations is that it is not recognized by switch recombination proteins but rather by a different DNA cleavage activity (translocase?). The latter enzyme may introduce a nick or gap into the DNA which is subsequently incorporated into the switch region. Note that the translocation breakpoints occur approximately one turn of the helix from the potential recognition sequence. It is unlikely that a free double-stranded DNA end is generated as an initial step in translocation because both segments of the *c-myc* gene remain linked to immunoglobulin sequences through a reciprocal exchange⁸. While sequence-specific DNA cleavage activity has been detected in some eukaryotic cells^{21,22}, we have as yet no evidence for this type of activity in plasmacytoma cells.

The question remains as to why the translocation breakpoints in mouse plasmacytomas nearly always truncate the untranslated first *c-myc* exon. There are many GAGG sequences throughout the region 5' to the first exon but no breakpoints have been mapped to this domain. Therefore, we assume that, in tumour formation, some additional selective advantage is conferred by removal of the first exon; this may be due to the presence of a repressor that binds to the 5' end of the gene²³ or the inability of an immunoglobulin heavy-chain enhancing element to increase *c-myc* RNA levels to a sufficiently high level while still using the normal promoter. Further studies will be required to elucidate the mechanism of chromosomal translocation and what influence the immunoglobulin locus has on expression of the *c-myc* oncogene.

Received 17 February; accepted 24 May 1984.

- Yunis, J. J. *Science* **221**, 227–236 (1983).
- Shen-Ong, G. L. C., Keath, E. J., Piccoli, S. P. & Cole, M. D. *Cell* **31**, 443–452 (1982).
- Taub, R. et al. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7837–7841 (1982).
- Crews, S., Barth, R., Hood, L., Prehn, J. & Calame, K. *Science* **218**, 1319–1321 (1982).
- Erikson, J., ar-Rushdi, A., Drwings, H. L., Nowell, P. C. & Croce, C. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 820–824 (1983).
- Marcu, K. B. et al. *Proc. natn. Acad. Sci. U.S.A.* **80**, 519–523 (1983).
- Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M. & Cory, S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1982–1986 (1983).
- Cory, S., Gerondakis, S. & Adams, J. M. *EMBO J.* **2**, 697–704 (1983).
- Harris, L. J., Lang, R. B. & Marcu, K. B. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4175–4179 (1982).

10. Calame, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6994–6998 (1982).
11. Stanton, L. W., Watt, R. & Marcu, K. B. *Nature* **303**, 401–406 (1983).
12. Bernard, O., Cory, S., Gerondakis, S., Webb, E. & Adams, J. M. *EMBO J.* **2**, 2375–2383 (1983).
13. Battey, J. *et al. Cell* **34**, 779–789 (1983).
14. Davis, M. M., Kim, S. K. & Hood, L. E. *Science* **209**, 1360–1365 (1980).
15. Word, C. J., Mushinski, J. F. & Tucker, P. W. *EMBO J.* **2**, 887–898 (1982).
16. Tucker, P. W., Slightom, J. L. & Blattner, F. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7684–7688 (1981).
17. Dunnick, W., Shell, B. E. & Dery, C. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7269–7273 (1983).
18. Neuberger, M. S. & Calabi, F. *Nature* **305**, 240–243 (1983).
19. Stanton, L. W. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 829–833 (1984).
20. Nikaïdo, T., Yamawaki-Kataoka, Y. & Honjo, T. *J. biol. Chem.* **257**, 7322–7329 (1982).
21. Rostriken, R., Strathern, J. N., Klar, A. J. S., Hicks, J. B. & Heffron, F. *Cell* **35**, 167–174 (1983).
22. Watabe, H., Ino, T., Kaneko, T., Shibata, T. & Ando, T. *J. biol. Chem.* **258**, 4663–4665 (1983).
23. Leder, P. *et al. Science* **222**, 765–771 (1983).
24. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499–560 (1980).
25. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
26. Adams, J. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6966–6970 (1982).

Apparent absence of transposable elements related to the P elements of *D. melanogaster* in other species of *Drosophila*

John F. Y. Brookfield*, Elizabeth Montgomery & Charles H. Langley

Laboratory of Genetics, National Institute of Environmental Health Sciences, PO Box 12233, Research Triangle Park, North Carolina 27709, USA

* Department of Genetics, School of Biological Sciences, University of Leicester, Leicester LE1 7RH, UK

P elements are transposable elements found in P strain, but usually not in M strain, *Drosophila melanogaster*, and are responsible for the hybrid dysgenesis that occurs when male *D. melanogaster* of the P strain mate with females of the M strain (ref. 1 and references therein). Several P elements, which vary in length and genetic effects, have now been cloned². To investigate the evolutionary origin of P elements, we have used a cloned copy of a *D. melanogaster* P element to look for related sequences in the genomes of six other *Drosophila* species. We report here that, unlike many other transposable elements found in *D. melanogaster*, which seem also to be present in other *Drosophila* species^{3–6}, we have found no sequences closely enough related to P elements to be detected by DNA hybridization in any other *Drosophila* species. This result supports the hypothesis that P elements have recently invaded *D. melanogaster* by horizontal transmission.

The discovery that most interspersed repetitive sequences in *D. melanogaster* are transposable⁷ raises important questions. It is still not known how transposable elements move to new sites in the genome, although the strong structural similarities emerging among vertebrate retroviruses and copia-like elements⁸ suggest that transposition of these elements may occur, analogously to retroviruses, via an RNA intermediate. Furthermore, while some insertions of transposable elements cause mutations of major effect^{9,10}, any effects of the vast majority of these elements on the host are not known. Third, how have transposable elements come to be in *Drosophila* genomes? Have they arisen through evolutionary changes generating new families of transposable elements within the *Drosophila* lineage, either from unique-sequence DNA or other families of transposable elements, or have *Drosophila* transposable elements been transmitted horizontally into *Drosophila* from other evolutionary lineages?

One approach to the question of the origin of transposable elements is to look for homologies between cloned middle repetitive sequences from *D. melanogaster* and the DNAs of related species. If these elements are capable of horizontal transmission between species, the distribution of such homology need not follow the phylogeny of the hosts, and might be more dependent, for example, on their geographical distributions. Several studies, including the present one, have addressed this question^{3–6}. With few exceptions, these experiments show that the presence or absence of homology between sequences in *D. melanogaster* and related species is distributed in a phylogenetic way.

To elucidate the origin of P elements, DNA from related species of *Drosophila* was extracted¹¹, digested with *Bam*HI, size-fractionated¹², blotted onto nitrocellulose¹³ and probed sequentially with three *D. melanogaster* sequences cloned into pBR322 and labelled with ³²P (ref. 14). The DNA used was derived from two M strains and one P strain of *D. melanogaster*, a yw laboratory stock of *Drosophila simulans*, three *D. simulans* isofemale lines recently extracted from a wild population in Raleigh, North Carolina, and wild-type laboratory stocks of *Drosophila mauritiana*, *Drosophila yakuba*, *Drosophila teissieri*, *Drosophila erecta* and *Drosophila pseudoobscura*. The sequences used as probes were p π 25.1 (containing an intact P element and flanking sequences), pS25.1 (containing only the flanking sequences (target site) from p π 25.1) and cDm2042 (containing the transposable element 412). As shown in Fig. 1, only in the P strain of *D. melanogaster* was homology to the P element found. The bands of hybridization in the other species were shown to be due to the *D. melanogaster* unique-sequence DNA on the P element probe. In contrast to the P element, multiple

Table 1 The distribution of homologies between 6 *Drosophila* species and 19 cloned middle repetitive sequences from *D. melanogaster*

Species	p π 25.1	cDm5002	cDm4006	cDm2042	pD75.3	cDm2161	cDm2217	cDm2244	cDm2157	cDm2154	cDm2179	cDm2186	cDm2210	cDm2218	cDm2228	cDm2156	cDm2173	cDm2181	cDm2219
<i>D. melanogaster</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>D. simulans</i>	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>D. mauritiana</i>	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>D. yakuba</i>	–	–	–	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+	+
<i>D. teissieri</i>	–	–	–	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+	+
<i>D. pseudoobscura</i>	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+

Species are listed in increasing phylogenetic distance from *melanogaster*. For more detail see ref. 19. The *D. melanogaster* DNA was extracted from an isofemale line, FM 115, from a natural population; the *D. simulans* DNA was from yw, a stock marked with the yellow and white mutations; remaining strains were obtained from the *Drosophila* Stock Center in Bowling Green, Ohio. p π 25.1 contains a complete P element flanked by unique-sequence DNA cloned into pBR322, cDm5002 contains copia, cDm4006 contains 297 and cDm2042 contains 412 (plasmids kindly supplied by G. M. Rubin and K. O'Hare)²⁰. pD75.3 is a cloned sequence in pBR322 containing a repetitive element from the foldback family²¹. The remaining 14 sequences were obtained from E. Strobel, and have been only partially characterized. They were isolated as middle repetitive sequences of 3–10 kilobases and have been classified as putative copia-like elements²². One of these elements, cDm2173, shows homology to the 'roo' (ref. 23) or B104 (ref. 24) element (C. Aquadro, personal communication). Techniques of DNA preparation, digestion with restriction enzyme, electrophoresis, blotting and hybridization were as described in Fig. 1 legend.

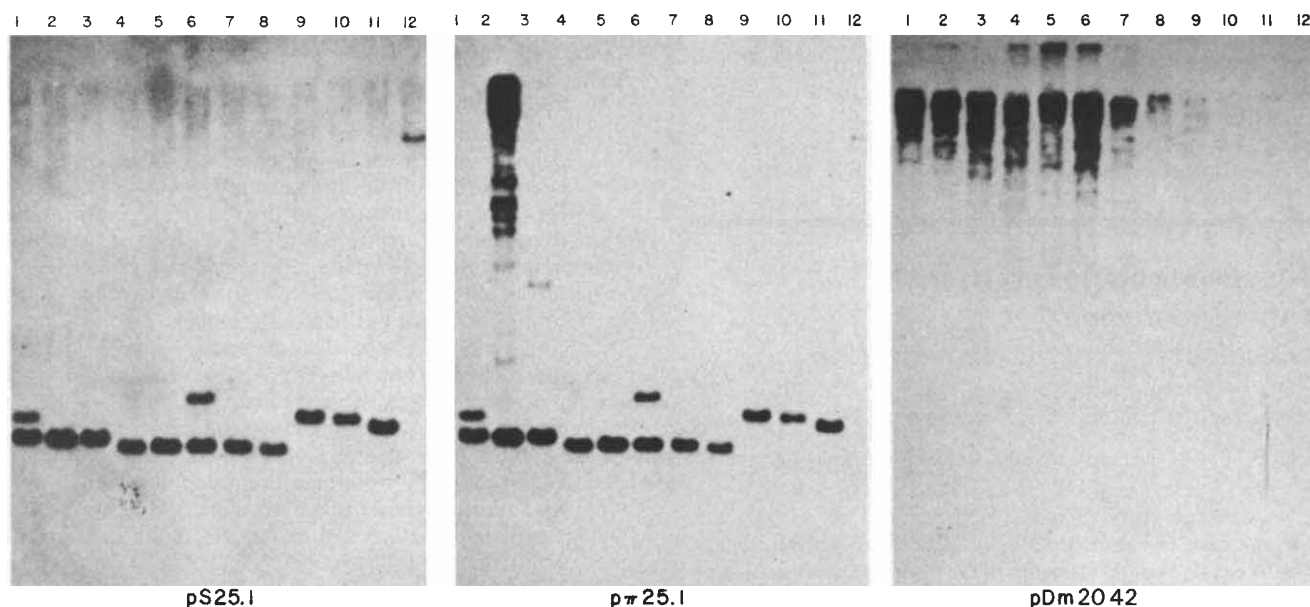


Fig. 1 Autoradiographs of a single Southern blot probed with three different DNAs. Species are listed in increasing phylogenetic distance from *D. melanogaster*. For more detail see ref. 19. Genomic DNAs were prepared, digested with *Bam*HI, electrophoresed, blotted and the filter dried. The nick-translation of DNA, hybridizations and washing of the filter were as described previously (refs 11–14) except that the hybridization temperature and post-hybridization wash were done at 37 °C for low stringency. The sources of the DNAs in the channels shown are as follows: 1, Oregon-R, an M strain (R. A. Voelker); 2, WE 7.29.12, a P strain (W. E. Engels); 3, C4, an M strain (R. A. Voelker); 4, FM1, *D. simulans* isofemale line; 5, FM2, *D. simulans* isofemale line; 6, FM3, *D. simulans* isofemale line; 7, *yw*, *D. simulans* marked with mutations *y* and *w* (P. M. Bingham); 8, *D. mauritiana*; 9, *D. yakuba*; 10, *D. teissieri*; 11, *D. erecta* (8–11 obtained from *Drosophila* Stock Center); 12, *D. pseudoobscura* (reference stock, F. Ayala). The filter was first probed with pS25.1, a single-copy *Bam*HI fragment from *D. melanogaster* in pBR322. After autoradiography, it was probed with pπ25.1, the same single-copy sequence as in pS25.1 which contains a complete P-element insertion in the central region (plasmids supplied by G. M. Rubin and K. O'Hare). After hybridization, the labelled probe was washed off the filter by treatment with 0.05% Denhardt's solution, 0.025% Na pyrophosphate, 2.5 mM Tris, 0.1 mM EDTA pH 8.0, for 3 h at 65 °C. The filter was then probed with cDm2042 which contains a complete 412 element flanked by unique-sequence DNA in pBR322. On longer exposure, the presence of multiple bands for 412 could be seen in lane 12 (*D. pseudoobscura*).

copies of all the other transposable elements from *D. melanogaster* were found in the two closest relatives of *melanogaster*, *D. simulans* and *D. mauritiana*, and many of the other transposable elements (for example, cDm2042, containing element 412) also showed homology to more distantly related species.

Table 1 shows the qualitative results from this study. The methods used were as above except that *Eco*RI, rather than *Bam*HI, was used to digest the genomic DNA, and the DNAs used comprised a subset only of those mentioned above. We also observed the conservation of internal restriction fragments in several of the elements. The presence or absence of these conserved fragments among the species also reflected phylogenetic relationships. In a similar study³, homology to *copia* was found to be distributed over a phylogenetically larger species group than our data indicate. This conflicting result may have been due to a difference in hybridization stringency. Our data show the presence or absence to follow absolutely the species phylogenetic relationship with *D. melanogaster*. Other studies show exceptions to this rule. Cases are known^{3,6} in which elements show a scattered distribution across the *Drosophila* genus. This could theoretically be due to horizontal transmission or to the extinction of transposable elements in certain lineages. Also, one study⁴ showed two elements which were present in *D. melanogaster* but absent in *D. simulans*, unlike any in our study. However, the absence of any hybridization at all to *D. simulans* DNA, despite the unique-sequence DNA which was present on the probes used, suggests that the hybridization conditions in this study may have been so stringent that a slightly diverged transposable element in *D. simulans* would not have been detected.

The data presented here suggest that while most transposable elements are ancient and stable components of the *Drosophila* genome, the P element appears to be a considerably more recent addition. This argues against the 'stochastic loss hypothesis'¹⁵,

which postulates that M strains were created by the random extinction by genetic drift of P elements in small populations of laboratory strains, resulting in a positive correlation between the age of a strain and its probability of being an M strain. This hypothesis also seems increasingly untenable due to the demonstration that only a minority of the cloned P elements are fully active P factors². This means that the generation of laboratory M strains by the stochastic loss of P factors would not necessarily be accompanied by the complete lack of P element homology which is the general case.

A more likely hypothesis is that in some way the P element has invaded *D. melanogaster* comparatively recently. Such an invasion may (in view of the variation in the frequencies of P and M cytotypes in strains collected at various recent dates and in various locations) have occurred in North America during the past 50 years¹⁶. The ability of P elements to transpose themselves into chromosomes when injected on plasmids into *D. melanogaster* embryos^{17,18} argues in favour of such transfer. However, until the vector in which the P element could have been introduced and the original host of the sequence are known, the invasion interpretation can be only tentative.

Received 16 January; accepted 22 May 1984.

- Engels, W. R. *A. Rev. Genet.* **17**, 315–344 (1983).
- O'Hare, K. & Rubin, G. M. *Cell* **34**, 25–35 (1983).
- Martin, G., Wiernasz, D. & Schedl, P. *J. molec. Evol.* **19**, 203–213 (1983).
- Dowsett, A. & Young, M. W. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4570–4574 (1982).
- Dowsett, A. P. *Chromosoma* **88**, 104–108 (1983).
- Young, M. W. & Schwartz, H. E. *Cold Spring Harb. Symp. quant. Biol.* **45**, 629–640 (1981).
- Young, M. W. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6274–6278 (1979).
- Shiba, T. & Saigo, K. *Nature* **302**, 119–124 (1983).
- Bingham, P. M. & Judd, B. H. *Cell* **25**, 704–711 (1981).
- Modolell, J., Bender, W. & Meselson, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1678–1682 (1983).
- Bingham, P. M., Levis, R. & Rubin, G. M. *Cell* **25**, 692–704 (1981).
- McDonnell, M. W., Simon, M. N. & Studier, F. W. *J. molec. Biol.* **110**, 119–146 (1977).
- Wahl, G. M., Stern, M. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683–3687 (1979).
- Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).
- Engels, W. R. *Cold Spring Harb. Symp. quant. Biol.* **45**, 561–565 (1981).
- Kidwell, M. G. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1655–1659 (1983).

17. Spradling, A. C. & Rubin, G. M. *Science* **218**, 341–347 (1982).
18. Rubin, G. M. & Spradling, A. C. *Science* **218**, 348–353 (1982).
19. Throckmorton, L. H. in *Handbook of Genetics* Vol. 3 (ed. King, R. C.) 421–469 (Plenum, New York, 1975).
20. Rubin, G. M. *et al.* *Cold Spring Harb. Symp. quant. Biol.* **45**, 619–628 (1981).
21. Levis, R., Collins, M. & Rubin, G. *Cell* **30**, 551–565 (1982).
22. Strobel, E. *Fedn Proc.* **41**, 2656–2658 (1982).
23. Meyerowitz, E. M. & Hogness, D. S. *Cell* **28**, 165–176 (1982).
24. Scherer, G., Tschudi, C., Perera, J., Delias, H. & Pirrotta, V. *J. molec. Biol.* **157**, 435–451 (1982).

Sequence-specific insertion of the *Drosophila* transposable genetic element 17.6

Satoshi Inouye*, Shunji Yuki* & Kaoru Saigo

Department of Biochemistry, Kyushu University 60 School of Medicine, Fukuoka 812, Japan

As in the case of retrovirus proviruses, most of the *Drosophila* copia-like transposable elements so far examined are bounded by 5'TG...CA3' and inserted into the chromosome without obvious site-specificity^{1–4}. In the other copia-like elements, 297, HMS Beagle and 17.6 (refs 5–7), terminal dinucleotides (5'TG...CA3') are completely absent and, instead, 5'(A)GT is present at least at one of the termini. One important feature of these three elements may be frequent insertion into 'TATA' boxes, since the three of four insertion sites so far examined were TATA boxes, two for H3 histone genes^{5,8} and one for a cuticle gene⁶. Because of the importance of this type of insertion, we extensively analysed site-specificity using 17.6 as a model. Our results, described here, suggest that insertion of 17.6 takes place in a site-specific fashion, using a target 5'ATAT corresponding to the major portion of the consensus TATA box⁹, TATA^AAT^A.

*Permanent addresses: Chisso Corporation, Japan (S.I.); Ishihara Sanyo Co. Ltd, Japan (S.Y.).

17.6 was originally identified as an ~7-kilobase (kb) insert in *Drosophila* histone genes¹⁰ and was shown⁷ to be a copia-like element closely related in evolution to 297. Using as a probe a 2.1 kb *Hind*III segment having no homology to 297 (see Fig. 1), 46 recombinant clones were isolated from 4×10^4 phages in a cloned library of *Drosophila melanogaster* DNA. Although deletions and artificial truncations with *Eco*RI were observed in 60% of the clones, mapping of the restriction enzyme sites followed by Southern hybridization (Fig. 1) showed that these recombinant clones as a whole represented the 17.6 elements located at 18 chromosomal loci corresponding to half of the total 17.6-insertion sites per haploid genome⁷.

Nucleotide sequences about the junctions between 17.6 elements and their flanking host DNAs were determined in 10 clones after subcloning suitable segments into pUC9 (Fig. 2)¹¹. The nucleotide sequences of the long terminal repeats (LTRs) of these 17.6 elements were very similar to each other, although deletion, insertion or substitution with considerable lengths were found in 4 out of 10 clones (data not shown). The results of a previous experiment⁷ suggested that a 17.6 found in a histone gene was bounded by 5'(A)GTG...CAAT(T)3', and 5'(T)ATAT(A) of the host DNA at the insertion site was duplicated on integration of 17.6 (Fig. 2a). The 1-base pair (bp) ambiguities at the very ends of 17.6 may be clarified by comparing the nucleotide sequences of the 17.6-host DNA junctions at different chromosomal loci. The results in Fig. 2b suggest that the 17.6 in λ KS514 terminates with 5'(A)GTG...CAATT3' and that its insertion results in the duplication of a host DNA sequence, ATAT(A). In contrast, the 17.6 in λ KS634 seems to end with 5'AGTG...CAAT(T)3', since the 17.6 is bordered by (T)ATAT and (T)ATATG at the left and the right sides, respectively (Fig. 2c). Although it is deleted (Fig. 1), the original left end of the 17.6 in λ KS525 is also expected to be 5'AGTG by similar reasoning (Fig. 2c). Thus, it may be concluded that 17.6 is generally bounded by 5'AGTG...CAATT3' and the size of its target host DNA is 4 bp.

Surprisingly, all targets of 17.6 elements so far examined were

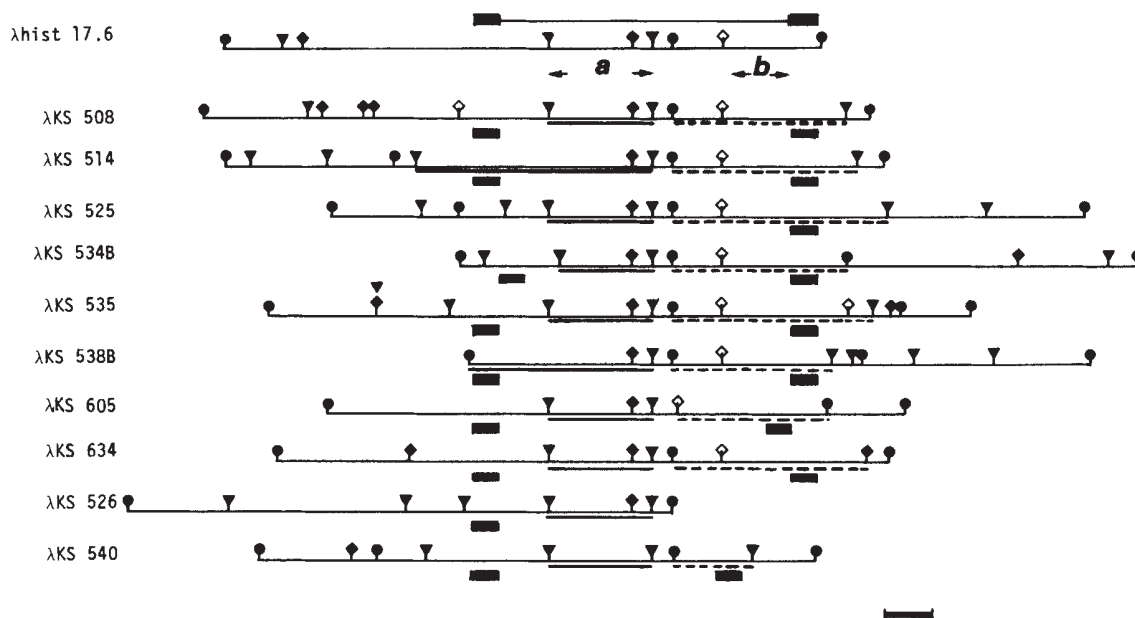


Fig. 1 Physical maps of representatives of *Drosophila* cloned segments containing 17.6 with that of the insert in λ hist17.6. The length and position of 17.6 in λ hist17.6 are schematically drawn over the map of λ hist17.6. Filled rectangles show the locations of the LTRs of each 17.6, as determined by fine mapping of the restriction sites or DNA sequencing. ●, *Eco*RI; ▼, *Hind*III; ◆, *Bam*HI; ◇, *Sal*I. The scale bar in the lower margin indicates 1 kb.

Methods: Using a 2.1-kb *Hind*III segment of λ hist17.6 (labelled a) as a probe, 46 positive clones were isolated from a cloned library¹⁰ of *Drosophila melanogaster* (Oregon R) DNA partially digested with *Eco*RI. After DNA was extracted, the physical map of each clone was constructed using *Eco*RI, *Hind*III, *Bam*HI and *Sal*I. Although the recombinant clones isolated were found to be composed of 18 different phages, only 10 representatives are shown here. DNA from recombinant phages was terminally digested with restriction enzymes, *Eco*RI and *Hind*III, denatured, neutralized, transferred to nitrocellulose papers and blot hybridized with suitable probes. Solid underlining shows the results when the 2.1-kb *Hind*III segment (labelled a) was used as a probe, and dashed underlining shows the segments hybridizable to the 1.5-kb *Pvu*II segment of 297 which is highly homologous to the region labelled b in λ hist17.6 (homology 90%).

ATAT, indicating that the insertion of 17.6 is carried out strictly in a site-specific fashion with ATAT as the target sequence. To our knowledge, there has been no report on the presence of eukaryotic transposable genetic elements or retrovirus proviruses exhibiting such a highly strict site-specificity as 17.6. The only possible exception is 297, a sibling of 17.6, since (1) Ikenaga and Saigo⁵ have suggested that at least within a histone gene cluster, the insertion of 297 is carried out in a site-specific fashion using (T)ATAT(A) as the target sequence, (2) the target sequences for integration of two 297 element, from *Drosophila simulans* and *Drosophila yakuba*, siblings of *D. melanogaster*, were found to be (T)ATAT(A) (data not shown), and (3) Young and Rubin suggested the possibility of the preferential insertion of 297 into ATAT (ref. 12). In this connection, it should be pointed out that 17.6 contains a 4.5-kb region whose homology to its counterpart of 297 is more than 70% (unpublished data).

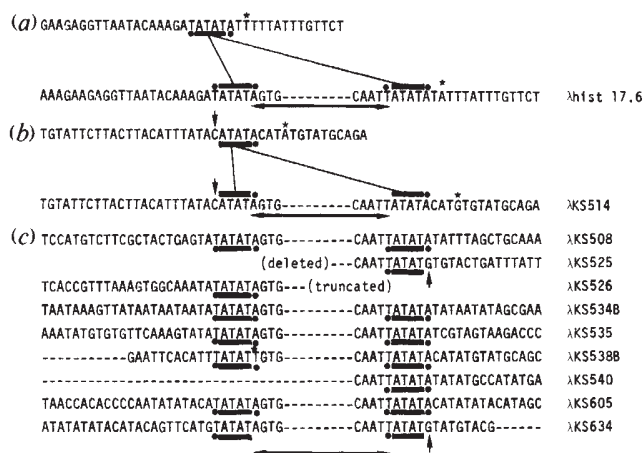


Fig. 2 The nucleotide sequences around the insertion sites of 17.6 transposable elements. The filled rectangles indicate the minimum sequences of the host targets which are expected to be duplicated on insertion of 17.6. Filled circles show the ambiguities in nucleotide sequence found at the ends of both host targets and 17.6 elements, whereas asterisks indicate base changes found near the insertion site of 17.6. *a*, 17.6-host DNA junctions in λ hist17.6⁷ (lower) and the corresponding empty site⁸ (upper). *b*, Relationship between a full (lower) and empty (upper) site for a 17.6 in λ KS514. *c*, The nucleotide sequences around the junctions between 17.6s and their flanking host DNAs in nine newly isolated clones. Upward arrow heads in λ KS525 and λ KS634 show the G residues which were used for delimitation of the 5' end of 17.6.

Methods: *a*, *b*, Using the right and left flanking sequences of the 17.6 in λ KS514 as probes, a recombinant clone containing the corresponding empty site was isolated from cloned library of *D. melanogaster* (Canton S)¹⁴. The nucleotide sequence around the empty site together with those for host DNA-17.6 junctions were determined according to Maxam and Gilbert¹⁵. Note the presence of C residues at the points labelled by arrowheads. DNA segments having the 17.6-host DNA junctions were cloned into pUC9 and the nucleotide sequences were determined by a procedure described by Maxam and Gilbert¹⁵.

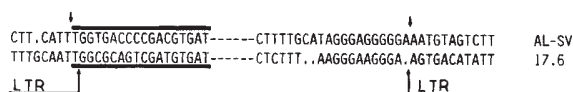


Fig. 3 The nucleotide sequences at the interval junctions of LTR of 17.6. The upper sequence is for the avian leukosis-sarcoma virus (AL-SV)²¹, while the lower one is for 17.6 (ref. 7). The correspondence of the base sequence between 17.6 and AL-SV is essentially the same as that previously described⁷. The L-shaped arrows labelled LTR indicate the LTRs of 17.6. Two downward arrows show the internal ends of LTRs of AL-SV DNA before integration. The thick line over the nucleotide sequence of AL-SV shows the primer binding site, whereas that of 17.6 indicates the putative primer binding site in 17.6.

Since the 4.5-kb region in 17.6 as well as that in 297 includes two open reading frames corresponding to polypeptides with molecular weights of 100,000 and 50,000, these coding regions may possibly be for a putative enzyme responsible for the site-specific integration of both 17.6 and 297. It is also noteworthy that 6 of the 11 target sites of 17.6 are localized within TATATAT, a canonical sequence of the TATA boxes⁹(TATA^AA^A), although at present, it is not understood what fractions of these sites can function as real TATA boxes.

The nucleotide sequence apparently corresponding to the primer binding site, which is mandatory for reverse transcription in a retrovirus system, has been recognized near one of the internal junctions of the LTRs of 17.6 (ref. 7). Furthermore, our recent analysis of the nucleotide sequence of 17.6 revealed that the amino acid sequence of a central portion (molecular weight in 30,000) of the polypeptide expected to be derived from the largest open reading frame of 17.6 is highly homologous to that of the counterpart of reverse transcriptase of Moloney murine leukaemia virus. Apparently, these findings provide positive evidence for the notion that 17.6 RNA is reverse-transcribable. Our sequence analysis presented here, however, shows that, unlike retroviruses¹³, in the case of 17.6 the left-most base of the putative primer binding site and the right-most base of the left-hand LTR overlap each other (Fig. 3). If a target for 297 is also ATAT, then the situation is the same in the case of 297 (ref. 7). Thus, it is suggested that even if 17.6 and 297 RNAs can be converted to DNA, the mechanism of reverse transcription of 17.6 and 297 must differ somewhat from those of retroviruses.

We thank K. Hattori for carrying out the restriction enzyme mapping. This work was supported by grants from the Ministry of Education, Science and Culture of Japan to K.S.

Received 10 May; accepted 4 June 1984.

1. Rubin, G. M. *et al.* *Cold Spring Harb. Symp. quant. Biol.* **45**, 619-628 (1981).
2. Kulguskin, V. V., Ilyin, Y. V. & Georgiev, G. P. *Nucleic Acids Res.* **9**, 3451-3463 (1981).
3. Will, B. M., Bayev, A. A. & Finnegan, D. J. *J. molec. Biol.* **153**, 897-915 (1981).
4. Scherer, G., Tschudi, C., Perera, J., Delius, H. & Pirrotta, V. *J. molec. Biol.* **157**, 435-451 (1981).
5. Ikenaga, H. & Saigo, K. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4143-4147 (1982).
6. Snyder, M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 7430-7434 (1982).
7. Kugimiya, W., Ikenaga, H. & Saigo, K. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3193-3197 (1983).
8. Goldberg, M. L. thesis, Stanford Univ. (1979).
9. Breathnach, R. & Chambon, P. *A. Rev. Biochem.* **50**, 349-383 (1981).
10. Saigo, K., Millstein, L. & Thomas, C. A. Jr *Cold Spring Harb. Symp. quant. Biol.* **45**, 815-827 (1981).
11. Vieira, J. & Messing, J. *Gene* **19**, 259-268 (1982).
12. Spradling, A. C. & Rubin, G. M. *A. Rev. Genet.* **15**, 219-264 (1981).
13. Swanson, R., DeLorbe, W. J., Bishop, J. M. & Varmus, M. E. *Proc. natn. Acad. Sci. U.S.A.* **78**, 124-128 (1981).
14. Maniatis, T. *et al.* *Cell* **16**, 687-701 (1978).
15. Maxam, A. M. & Gilbert, W. *Methods Enzym.* **65**, 499-560 (1980).

Conservation of the sizes for one but not another class of exons in two chick collagen genes

Yoshihiko Yamada*, Gene Liau, Maria Mudryj, Silvana Obici* & Benoit de Crombrughe

Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205, USA

Type III collagen is often found in the same tissues as type I collagen, yet the function and nature of the fibrils formed by the two collagens differ markedly¹⁻³. To understand the evolutionary history of the collagen gene family in more detail, we isolated the gene for type III collagen and compared its structure with that of the gene for $\alpha 2(I)$ collagen⁴⁻⁹. This comparison points to a remarkable conservation in the size distribution of the exons coding for the helical part of these two collagen polypeptides: equivalent amino acid segments in the helical domain of each polypeptide

* Present addresses: Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, Bethesda, Maryland 20205, USA (Y.Y.); Institute of General Pathology, 2nd Medical School, University of Naples, Italy (S.O.).

are encoded by exons of equal sizes in each gene. This suggests that after the interstitial collagen genes had been duplicated from a common ancestor about $2-5 \times 10^8$ years ago¹⁰, no recombinations between these exons were tolerated, although the same recombinational phenomena must have played an important part in shaping the structure of the progenitor for these genes. This fixation of the size distribution of the exons which code for the interstitial collagen helical domains is found despite the persistence in these exons of sequence elements that should have favoured recombinational rearrangements, and contrasts with the variations in the pattern of sizes of some exons coding for the amino and carboxyl propeptides of these collagens.

We previously reported the isolation of a genomic clone C3-C1-24 which contains the 3' portion of the chick $\alpha 1(\text{III})$ collagen gene¹¹. We have now isolated a series of overlapping clones which cover about 60 kilobases (kb) of contiguous DNA sequences in the chick genome and span almost the entire gene (Fig. 1). Figure 2 shows a diagrammatic representation of the structure of the $\alpha 1(\text{III})$ collagen gene derived from measurements of R-loop electron microscopy data obtained by hybridizing each clone with a preparation of $\alpha 1(\text{III})$ collagen mRNA. The $\alpha 1(\text{III})$ collagen gene is at least 35 kb long and contains more than 49 exons. The precise location of the 3' end of the gene was previously determined by DNA sequencing¹². We have

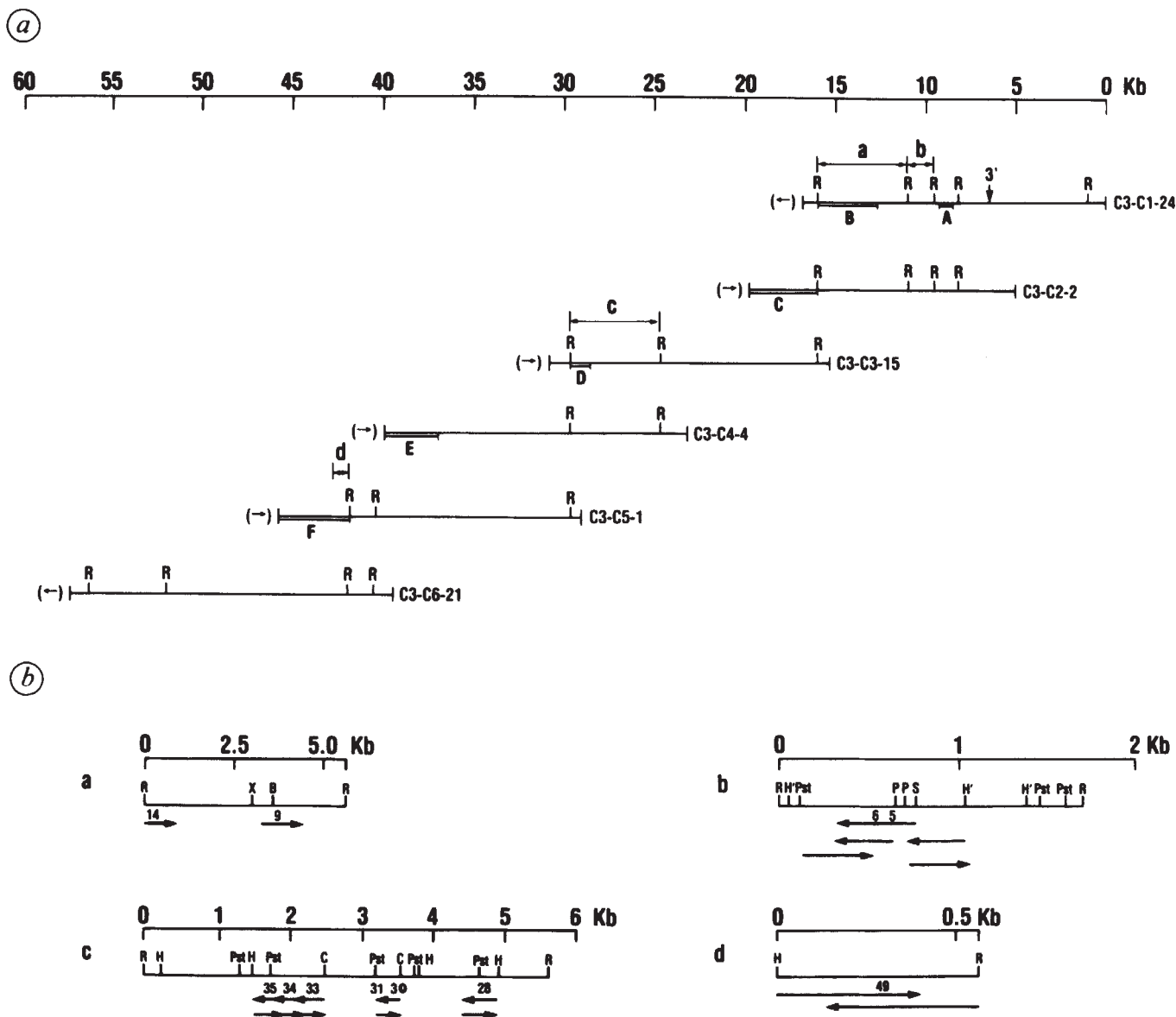


Fig. 1 Overlapping clones of the chick type III collagen gene and maps of fragments used for DNA sequence analysis. *a*, A genomic library (gift from Dr S. Dodgson) containing fragments of a partial *AluI*-*HaeIII* digestion of chick DNA cloned into the λ Charon 4A vector, was screened as described previously³¹. The isolation of the first clone has been reported previously¹¹. It was isolated by cross-hybridization with an $\alpha 1(\text{I})$ collagen cDNA clone³² shown as segment A. Subsequent clones were isolated by successive screening of the library using as hybridization probes the genomic DNA fragments marked B, C, D, E and F. R indicates *EcoRI* sites. Horizontal arrows pointing to the right indicate that the 5'→3' orientation of the type III collagen gene sequences is the same as the conventional 5'→3' orientation of the λ map. Horizontal arrows pointing to the left indicate that the orientation of the collagen gene sequences is opposite to that of the λ map. *b*, Arrows indicate the direction of sequencing. The numbers above the arrows represent exon numbers. The DNA fragments were labelled with ³²P at either their 5' or 3' end by T4 kinase or terminal transferase, respectively. Nucleotide sequences were determined as described by Maxam and Gilbert³³. *a*, 5.5-kb *EcoRI* fragment of C3-C1-24; *b*, 1.7-kb *EcoRI* fragment of C3-C1-24; *c*, 5.7-kb *EcoRI* fragment of C3-C3-15; *d*, 0.6-kb *HindIII*-*EcoRI* fragment of C3-C5-1. B, C, H, H', P, Pst, S and X indicate the restriction enzymes *Bam*HI, *Cfo*I, *Hind*III, *Hinf*I, *Pvu*II, *Pst*I, *Sac*II and *Xba*I, respectively.

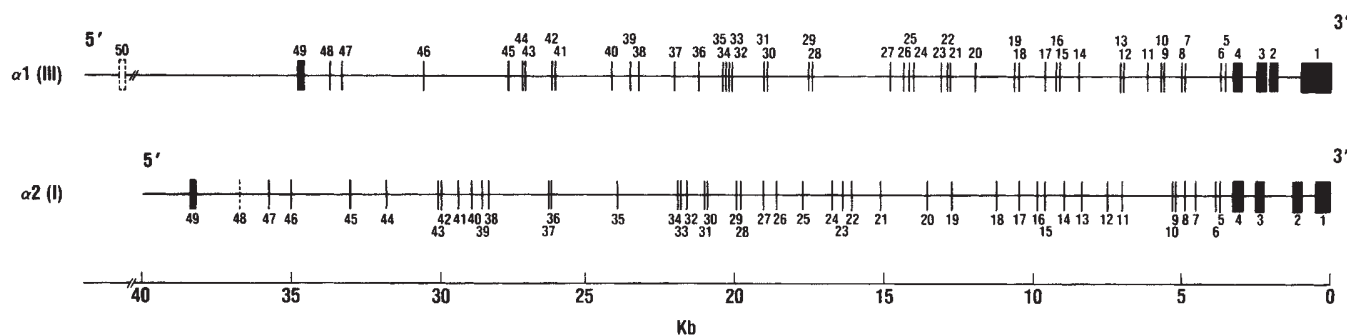


Fig. 2 Schematic comparison of the structures of the $\alpha 1(\text{III})$ and $\alpha 2(\text{I})$ collagen genes. Vertical bars represent exons. Horizontal lines between vertical bars indicate introns. The sizes of the introns were determined by electron microscopic analysis of R-loops between each of the recombinant DNAs shown in Fig. 1 and a preparation enriched for chick type III collagen RNA. DNAs from each of the genomic clones ($5 \mu\text{g ml}^{-1}$) and from Charon 4A ($5 \mu\text{g ml}^{-1}$) were heat-denatured and mixed with chick embryo crop poly(A) RNA ($3 \mu\text{g}$ in $20 \mu\text{l}$) in 50% formamide, 0.1 M Tricine pH 8.0 and 0.5 M NaCl for 3 h at 52°C . Samples were mounted for electron microscopy as described elsewhere³⁴. Double-stranded circular simian virus 40 DNA was used as an internal size standard. The structure of the 3' part of the gene was reported previously¹¹. The size of the overlaps between clones was determined by measurements of heteroduplexes between two overlapping clones of similar polarity. The number of exons is based on careful examination of multiple R-loop electron micrographs; one cannot exclude the possibility that very small introns were overlooked by this method. Exons 1 to 4 code for the carboxy-propeptide extension of $\alpha 2(\text{I})$ and $\alpha 1(\text{III})$ collagen. Exon 49 of $\alpha 1(\text{III})$ collagen codes for the amino terminal of the amino propeptide. Exon 49 of $\alpha 2(\text{I})$ collagen corresponds to the 5'-untranslated region and the signal peptide sequence. Note that the numbering of a few of the exons in the $\alpha 2(\text{I})$ collagen gene has changed compared with previous publications from this laboratory^{6,7}.

Exon number		Size of exon (bp)	Amino acid sequence homology	
			Human	Calf
49	cctag GA GGA TGT ACC CAT CTT GGG CAG GAA TAT GCT GAC AGG GAT GTT TGG AAA CCA Gly Gly Cys Thr His Leu Gly Gln Tyr Ala Asp Arg Asp Val Trp Lys Pro GAG CCA TGC CAA ATC TGT GTG TGT GAC TCT GGA TCT GTT CTC TGT GAT GAC ATC Glu Pro Cys Gln Ile Cys Val Cys Asp Ser Gly Ser Val Leu Cys Asp Asp Ile ATC TGT GAT GAT CAG GAG TTG CAT TGT CCC AAC CCA GAG ATT CCC CTC GGA GAA Ile Cys Asp Asp Gln Glu Leu Asp Cys Pro Asn Pro Glu Ile Pro Leu Gly Glu TGC TGT CCA GTT TGC CCG CAG ACA ACA CCA CAG CCT ACA AAA gtaagt Cys Cys Pro Val Cys Pro Gln Thr Thr Pro Gln Pro Thr Arg	203		87%
35	ttaag GGT CCT CCA GGT CCC CCT GGA ACT GCA GGA TTT CCG GGC TCT CCA GGT TTT AAG gatttt Gly Pro Pro Gly Pro Pro Gly Thr Ala Gly Phe Pro Gly Ser Pro Gly Phe Lys 177	54	94%	94%
34	cctag GGT GAA GCT GGT CCT CCT GGC CCT GCT GGT GCT AGT GGC AAT CCT GGT GAG AGA Gly Glu Ala Gly Pro Pro Gly Pro Ala Gly Ala Ser Gly Asn Pro Gly Glu Arg 178 GGA GAA CCT GGT CCT CAG GGT CAA GCT GGC CCC CCT GGC CCT CAG gtacgg Gly Glu Pro Gly Pro Gln Gly Gln Ala Gly Pro Pro Gly Pro Gln 210	99	67%	70%
33	tccag GGT CCT CCT GGA AGA GCT GGA AGC CCT GGC GGC AAG GGT GAA ATG gtaagt Gly Pro Pro Gly Arg Ala Gly Ser Pro Gly Gly Lys Gly Glu Met 225	45	87%	87%
31	tttag GGA GAA CCT GGT AAG AAT GGT GCA AAA GGA GAT CCA GGC CCA AAA GGC GAG CGT gtaagt Gly Glu Pro Gly Lys Asn Gly Ala Lys Gly Asp Pro Gly Pro Lys Gly Glu Arg 259	54	89%	94%
30	gccag GGT GAA AAT GGC ACC CCA GGT GCG CGT GGA CCT CCC GGA GAG GAA GGC AAG AGA Gly Glu Asn Gly Thr Pro Gly Ala Arg Gly Pro Pro Gly Glu Glu Gly Lys Arg 277 GGT GCA AAC GGT GAA CCT GGT CAG AAT GGC GTT CCT GGC ACA CCC GGA GAG AGG gtaaac Gly Ala Lys Gly Glu Pro Gly Arg Lys Gly Val Pro Gly Thr Pro Gly Glu Arg 312	108	61%	61%
28	tttag GGT CCT GCA GGT GAG CGT GGC AGC CCA GGT CCC CCT GGC CCA AGT GGA CCT GCG Gly Pro Ala Gly Glu Arg Gly Ser Pro Gly Pro Pro Gly Pro Ser Gly Pro Ala 331 GGA GAC CGT GGT CAA GAT GGA GGC CCA GGT CTT CCA GGA ATG AGG gtaaga Gly Asp Arg Gly Gln Asp Gly Gly Pro Gly Leu Pro Gly Met Arg 363	99	72%	66%
14	tgtag GGT GCT GCT GGT TTC CCT GGT GCT CGT GGT CCA CCC GGT CCC CCT GGT AAT AAT gtaagt Gly Ala Ala Gly Phe Pro Gly Ala Arg Gly Pro Pro Gly Pro Pro Gly Asn Asn 697	54	89%	89%
9	tgtag GGT GAC CGT GGT GAA AGT GGC CCT CCT GGT GTT CCA GGA CCT CCT GGT CAC CCA Gly Asp Arg Gly Glu Ser Gly Pro Pro Gly Val Pro Gly Pro Pro Gly His Pro 859 GGG CCT GCA GGT AAC AAC GGA GGC CCT GGA AAA GCT GGT GAA AGA GGA TTT CAG gtaaaa Gly Pro Ala Gly Asn Asn Gly Ala Pro Gly Lys Ala Gly Glu Arg Gly Phe Gln 894	108	67%	67%
6	tgtag GGT CCC CTA GGT CCT CAA GGT GCA ATT GGA AGT CCA GGA GCT TCA GGA GCA AGG gtaagg Gly Pro Leu Gly Pro Gln Gly Ala Ile Gly Ser Pro Gly Ala Ser Gly Ala Arg 949	54	72%	72%
5	tctag GGC CCA CCC GGA CCA GCT GGC CCC CCT GGA AAA GAT GGT AGA GGT GGC TAC CCA Gly Pro Pro Gly Pro Ala Gly Pro Pro Gly Lys Asp Gly Arg Gly Gly Tyr Pro 967 GGT CCA ATT GGT CCT CCT GGC CCG CGG GGT AAC AGA GGT GAA AGT GGC CCT CCA gtaagt Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly Asn Arg Gly Glu Ser Gly Pro Ala 1002	108	78%	78%

Fig. 3 Nucleotide sequence of various exons of the type III collagen gene. Numbers beneath the amino acid sequence correspond to the residue numbers in human type III collagen³⁵. Underlined nucleotide sequences indicate splicing signals. By comparing the amino acid sequences deduced from the exon sequences with the known amino acid sequences of human and calf $\alpha 1(\text{III})$ collagen, we were able to assign the sequences encoded by the exons to specific amino acid segments in the $\alpha 1(\text{III})$ polypeptide.

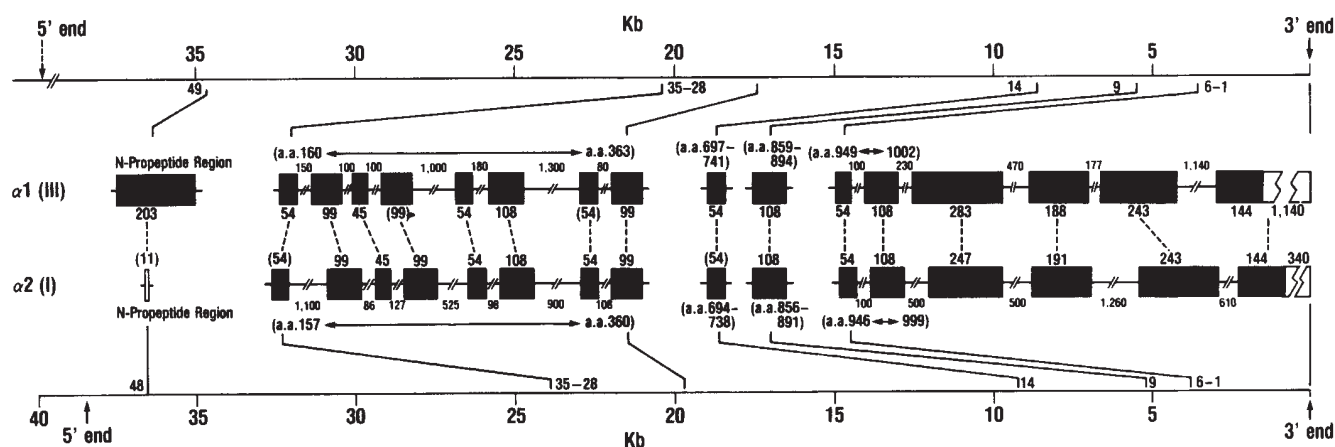


Fig. 4 Comparison of the pattern of exon sizes in the type III and $\alpha 2$ type I collagen genes. Boxes represent exons: open boxes, 3'-untranslated segments. The numbers immediately under or above the boxes indicate exon sizes in bp; those between parentheses were not obtained from the nucleotide sequence but were deduced. Numbers between exons represent intron sizes in bp. Numbers preceded by a.a. represent amino acid residue numbers in the protein sequence. The upper and lower line represents the $\alpha 1$ (III) and $\alpha 2$ (I) gene, respectively. The location of the exons that were examined within these genes and their numbers (as noted in Fig. 2) are indicated. The helical domain of human and calf $\alpha 1$ (III) collagen contains three more residues at the amino-terminal end than the helical domain of $\alpha 2$ (I) collagen. This explains why the amino acid residue numbers of $\alpha 1$ (III) collagen differ by three from those of $\alpha 2$ (I) collagen. We also noted that three amino acids present at the carboxy-terminal part of the helical domain of the $\alpha 1$ (III) gene in humans are not found in the chick $\alpha 1$ (III) collagen.

not yet determined the exact location of the 5' end of the gene but have evidence that a very large intron separates the 5' most proximal exon of the $\alpha 1$ (III) collagen gene and exon 49 (M.M., unpublished results). The DNA sequence of exon 49 indicates that this exon contains 203 base pairs (bp) and codes for the amino-terminal portion of the N-propeptide. From the sequence of a cDNA corresponding to the 5' end of this mRNA¹³, one can deduce that there must be at least one additional exon located 5' to exon 49—this exon should contain the information for the 5'-untranslated region, the signal peptide and also for the first $5\frac{1}{2}$ residues of the aminopropeptide of $\alpha 1$ (III) collagen.

In $\alpha 2$ (I) genes in the chick^{6,8,9} and mouse¹⁴ and in human $\alpha 1$ (I) (see accompanying paper¹⁵) and the chick $\alpha 1$ (II) collagen genes (W. Upholt, personal communication), exons which code for the helical domain of the protein all fall in a small number of very discrete size classes. The majority of exons are 54 bp long, whereas the other exons have sizes of 45, 99, 108 and 162 bp. These studies suggested (1) that the ancestor for these collagen genes had been assembled by amplification of a genetic unit containing an exon of 54 bp embedded in intron sequences⁶, (2) that exons which are shorter or longer than 54 bp presumably arose from secondary recombinational rearrangements between 54-bp exons, and (3) that the relative frequency of the exons which are shorter or longer than 54 bp was a reflection of the many secondary recombinational rearrangements of exons that occurred during or after the assembly of the ancestor for the interstitial collagen genes.

To determine whether exons containing 54, 45, 99 and 108 bp in the $\alpha 2$ (I) gene have the same length at identical positions in the $\alpha 1$ (III) collagen gene, we determined the nucleotide sequence of 10 exons coding for the helical domain of $\alpha 1$ (III) collagen, and of one exon for the globular domain of the aminopropeptide (Fig. 3). The DNA sequence of the four exons coding for the carboxyl propeptide was determined previously¹². Exons 35, 31, 14 and 6 contain 54 bp, exons 30, 9 and 5 have 108 bp, exons 34 and 28 contain 99 bp, and exon 33 is 45 bp long. We can also deduce that exon 29 has 54 bp and that exon 32 has 99 bp, as each of these two exons lies between a pair of exons for which the sequence was determined. As in the other interstitial collagen genes, the first codon of each exon is always a glycine triplet and the last codon is the one preceding a glycine codon. Exons 1, 2, 3 and 4 which encode the C-terminal propeptide contain, respectively, 1,300, 243, 188 and 283 bp¹¹.

Figure 4 shows that the pattern of sizes for the exons coding

for the helical domain of the polypeptide are precisely conserved in the $\alpha 1$ (III) and $\alpha 2$ (I) collagen genes, although the sequences at these exons vary considerably. Equivalent amino acid segments that are encoded by exons of a given length in one gene are also encoded by exons of identical length in the other gene. In contrast to the conservation of the sizes of the exons coding for the helical domain of the polypeptide, the sizes of the exons coding for the N-propeptide and the C-propeptide are not as well conserved. Exon 1 of the $\alpha 1$ (III) gene is larger than exon 1 of the $\alpha 2$ (I) gene because of a much larger 3'-untranslated sequence, the length of the coding region of exon 1 being the same in the two genes^{8,10}. Exon 3 has 3 bp less in the $\alpha 1$ (III) gene. Exon 4 has 36 bp less in the $\alpha 2$ (I) gene than in the $\alpha 1$ (III) gene. Exon 49 is much larger in the gene for $\alpha 1$ (III) than the equivalent exon in the gene for $\alpha 2$ (I) collagen¹⁶. Other elements of these genes have also changed: except for the splice signals, the sequences and sizes of the introns are very different. There are also several repetitive sequences in each gene. Each gene contains a member of the CR₁ repetitive family¹⁷, but these are found in two very different places in the two genes, and their sequences, although related, are not very similar (R. Frunzio, personal communication). What emerges, therefore, from a comparison of the structure of these two genes, is a rigid conservation in the pattern of sizes of the exons coding for the helical segments of the proteins, in contrast to changes in the sizes of some of the other exons and a large range of variations in all other elements of these genes.

Although the positions and the number of introns are conserved among the members of many other multigene families (the actin gene family is an exception to the rule), the sizes of equivalent exons are often not strictly conserved in different members of a family¹⁸⁻²⁸.

The strict conservation of the pattern of sizes for exons encoding the helical domains of two chick collagens suggests that the presumed recombinational rearrangements of exons that occurred during or after the assembly of the ancestral gene, were no longer tolerated after the two genes had been duplicated from a common ancestor, despite the presence in these exons of conserved sequence elements that should have favoured recombinational rearrangements (that is, repeating glycine triplets often associated with proline codons). These presumed recombinational rearrangements include unequal crossings-over between 54-bp exons to generate 45- and 99-bp exons, and fusions of 54-bp exons by recombination between the gene and

a cDNA intermediate^{29,30} to generate 108-bp and 162-bp exons. At one time in evolution during the assembly of the progenitor for the interstitial collagen genes, the sizes of the exons coding for the helical domain of the polypeptide became fixed, whereas many other elements in this gene continued to drift. It is unlikely that the uniformity in the pattern of exon sizes which we have observed was achieved by more recent phenomena related to gene conversion, because the sequences of a given exon are, in general, more analogous to the equivalent exon in the same gene in another species than to any other exon. The complete divergence of the intron sequences and the presence of repetitive sequences at different locations in these two genes are consistent with this notion. The fixation of the size of exons may have corresponded to the acquisition of an optimal length for the collagen molecule, although one has to assume that at least some of the preceding exon amplification steps and some of the previous recombinational rearrangements of exons, both of which changed the size of the collagen polypeptide, also provided an evolutionary advantage to the species in which it occurred. Reaching a functionally optimal length meant that the size of the helical domain of the collagen polypeptide could not be altered although changes in the sizes of the N- and C-peptide took place. A probable determining factor in optimizing the length of the collagen chain can probably be found in the highly structured fibrils of the different interstitial collagens which are the result of the interactions between individual triple helical collagen molecules that overlap each other in a regular and repeating pattern. Shorter or longer polypeptide chains which would be generated by recombination between exons could be expected to impede the orderly formation of these highly organized fibrils.

The above arguments do not explain why adjacent exons were not fused together more often by the elimination of intervening introns, a rearrangement that would not result in changes in the size of the polypeptide. The occurrence of several 108-bp exons suggests that such fusions occurred during or after the assembly of the ancestral gene. A possible explanation is based on the hypothesis that in order to convert the primary transcription products of these genes to a mature, translatable messenger RNA, a uniquely ordered succession of splicing reactions must take place, ensuring that the correct splicing partners are juxtaposed each time a splicing reaction occurs so that the 50 or so introns are precisely eliminated from the RNA precursor without the removal of exon sequences. During the formation of the gene an increasingly complex splicing pattern was probably engendered, characterized by a unique succession of splicing reactions. Removal of one or more introns in the DNA might cause a change in this succession of splicing reactions and produce an aberrant pattern of juxtaposition of splicing partners.

The rigid structural features of the interstitial collagen molecule itself are paralleled by a rigid evolutionary history which maintained the sizes of the exons, in contrast with the higher flexibility which has characterized the evolution of the globular domains of the collagen polypeptides. In general, rigid structural proteins may probably be expected to have undergone a much more conservative evolution than more flexible globular polypeptides.

12. Yamada, Y., Kuhn, K. & de Crombrughe, B. *Nucleic Acids Res.* **11**, 2733–2744 (1983).
13. Yamada, Y., Mudryj, M. & de Crombrughe, B. *J. biol. Chem.* **258**, 14914–14919 (1983).
14. Munson, J. M. & McCarthy, B. J. *DNA* **1**, 59–69 (1982).
15. Mon-Li Chu *et al.* *Nature* **310**, 337–340 (1984).
16. Tate, V. E., Finer, M. H., Boedtker, H. & Doty, P. *Nucleic Acids Res.* **11**, 91–104 (1983).
17. Stumph, W. E., Kristo, P., Tsai, M. J. & O'Malley, B. W. *Nucleic Acids Res.* **9**, 5383–5397 (1981).
18. Elstradatis, A. *et al.* *Cell* **21**, 653–668 (1980).
19. Eiferman, F. A., Young, P. R., Scott, R. W. & Tilghman, S. M. *Nature* **294**, 24–31 (1981).
20. Heilig, R., Perrin, F., Gannon, F., Mandel, J. L. & Chambon, P. *Cell* **20**, 625–637 (1980).
21. Royal, A. *et al.* *Nature* **279**, 125–132 (1979).
22. Yamawaki-Kataoka, Y., Miyata, T. & Honjo, T. *Nucleic Acids Res.* **9**, 1365–1381 (1981).
23. Wahli, W., David, I. B., Wyler, T., Weber, R. & Ryffel, G. V. *Cell* **20**, 107–117 (1980).
24. Nishioka, Y. & Leder, P. *Cell* **18**, 875–882 (1979).
25. Konkel, D. A., Tilghman, S. M. & Leder, P. *Cell* **15**, 1125–1132 (1978).
26. Patient, R. K., Elkington, J. A., Kay, R. M. & Williams, J. G. *Cell* **21**, 565–573 (1980).
27. Rabbitts, T. H., Forster, A. & Milstein, C. P. *Nucleic Acids Res.* **9**, 4509–4524 (1981).
28. Sargent, T. D., Jagodzinski, L. L., Yang, M. & Bonner, J. *Molec. cell. Biol.* **1**, 871–883 (1981).
29. Nishioka, Y., Leder, A. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2806–2809 (1980).
30. Wilde, C. D., Crowther, C. E. & Cowan, N. J. *Science* **217**, 549–552 (1982).
31. Maniatis, T. *et al.* *Cell* **15**, 687–701 (1978).
32. Yamamoto, T. *et al.* *J. biol. Chem.* **255**, 2612–2615 (1980).
33. Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).
34. Tilghman, S. M., Curtis, P. J., Tiemeier, D. C., Leder, P. & Weissman, C. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1309–1313 (1978).
35. Sayer, J. M. & King, A. H. *Biochemistry* **20**, 2621–2617 (1981).

Human pro α 1(I) collagen gene structure reveals evolutionary conservation of a pattern of introns and exons

Mon-Li Chu*, Wouter de Wet†, Michael Bernard*†, Juy-Fang Ding*, Maria Morabito†, Jeanne Myers*, Charlene Williams* & Francesco Ramirez*†

* Department of Biochemistry and † Department of Obstetrics and Gynecology, University of Medicine and Dentistry of New Jersey—Rutgers Medical School, Piscataway, New Jersey 08854, USA

‡ Department of Biochemistry, Potchefstroom University, 2520 Potchefstroom, South Africa

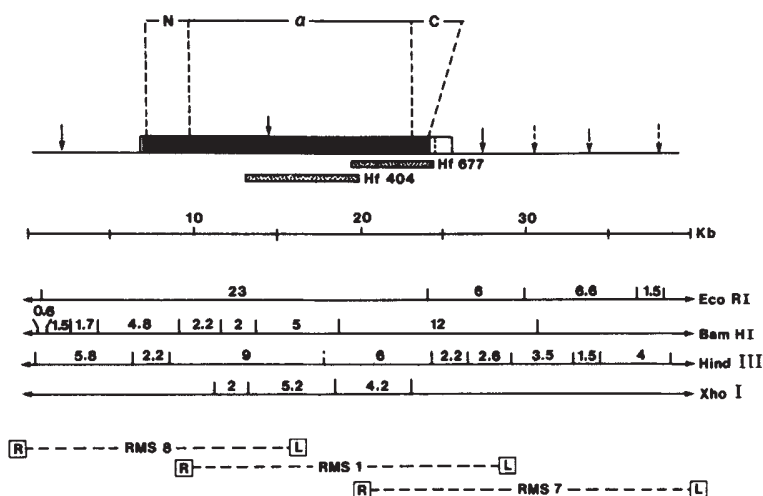
The collagens represent an interesting example of a structurally related but genetically distinct family of proteins¹. Type I, the most abundant of the vertebrate collagens, comprises two pro α 1(I) chains and one pro α 2(I) chain, each containing terminal propeptides and a central domain of 338 (Gly, X, Y) repeats. The structure of the chicken pro α 2(I) gene shows an intriguing relationship between exon organization and the arrangement of (Gly, X, Y) repeats (see ref. 2 for review). This has led to the suggestion³ that the collagens evolved from a common ancestral unit of 54 base pairs (bp). Here we present the structure of the entire human pro α 1(I) gene and compare this with the chicken pro α 2(I). The exon arrangement of the two genes is remarkably similar, although the human pro α 1(I) is more compact because of the shorter length of its introns. The data strongly support the notion that the type I genes have evolved from an ancestral multi-exon unit, and that once the gene was translated, a strong evolutionary pressure caused it to maintain this elaborate structure.

The human pro α 1(I) collagen gene was isolated within three overlapping genomic clones (Fig. 1). The gene is interdispersed by 50 introns, resulting in a highly complex structure, remarkably similar to that of the chicken pro α 2(I) gene (Fig. 2). However, whereas the intron sequences of the pro α 2(I) constitute 34 kilobase pairs (kbp) or 90% of the total gene size (39 kbp)², the combined length of the pro α 1(I) introns is 12 kbp or 70% of the total gene size (18 kbp). Thus, the great disparity in the lengths of the type I genes is primarily due to the size of their introns, which otherwise are localized in homologous positions. Forty-one exons consisting of 54 or a multiple of 54, code for 996 amino acid residues of the triple helical domain of the pro α 1(I) gene. Comparison with the avian gene (H. Boedtker, personal communication) revealed only one major difference: amino acid residues 568–603 are encoded by a single 108-bp exon (number 19) in the pro α 1(I) gene and by two 54-bp exons in the pro α 2(I) gene. A similar picture of structural complexity

Received 13 February; accepted 17 May 1984.

1. Remachandran, G. N. & Reddi, A. H. (eds) *Biochemistry of Collagen* (Plenum, New York, 1980).
2. Bornstein, P. & Sage, H. A. *Rev. Biochem.* **49**, 954–1003 (1980).
3. Miller, E. J. & Gray, S. *Meth. Enzym.* **82**, 3–32 (1982).
4. Ohkubo, H. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **77**, 7059–7063 (1980).
5. Wozney, J., Hanahan, D., Tate, V., Boedtker, H. & Doty, P. *Nature* **294**, 129–135 (1981).
6. Yamada, Y. *et al.* *Cell* **22**, 887–892 (1980).
7. Vogeli, G. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **78**, 5334–5338 (1981).
8. Dickson, L. A. *et al.* *J. biol. Chem.* **256**, 8407–8415 (1981).
9. Tate, V., Finer, M., Boedtker, H. & Doty, P. *Cold Spring Harb. Symp. quant. Biol.* **47**, 1039–1049 (1982).
10. Dickerson, R. E. *J. molec. Evol.* **1**, 26–45 (1971).
11. Yamada, Y., Mudryj, M., Sullivan, M. & de Crombrughe, B. *J. biol. Chem.* **258**, 2758–2761 (1983).

Fig. 1 Composite restriction endonuclease map of the human pro $\alpha 1(I)$ collagen gene clones RMS-1, RMS-7 and RMS-8. The upper half shows 40 kbp of genomic DNA containing the pro $\alpha 1(I)$ gene (solid box) and its relationship with the three domains of the protein molecule: amino-terminal prepropeptide (N), α -chain (α) and carboxy-terminal (C). The open boxes represent the 5' and 3' noncoding regions. The dotted line in the latter indicates the end of the smaller polymorphic mRNA transcript¹⁸, the exact 5' and 3' ends of the gene were determined by S₁ nuclease mapping in conjunction with DNA sequencing (M.-L. Chu *et al.*, in preparation). The solid arrow indicates the position of short repetitive sequences; dotted arrows indicate the approximate position of other repetitive sequences of undetermined size. Two of these repetitive elements were sequenced and found to be different members of the *Alu*I family. Similarly to the germline elements of the human pro $\alpha 2(I)$ collagen gene¹⁸ one of the pro $\alpha 1(I)$ *Alu* repeats is localized 3 kbp downstream from the termination codon and the other is found 12 kbp upstream within the second largest intron (XXVI) of the gene. Underneath is shown the region of the pro $\alpha 1(I)$ gene covered by two cDNA clones (Hf-404 and Hf-677)¹⁹ used for its isolation. At the bottom are shown the relative positions of the three overlapping genomic clones and their orientation within the left (L) and right (R) arms of the Charon 4A vector.



is emerging for the other fibrillar collagen genes, pro $\alpha 1(II)$ ⁴ and pro $\alpha 1(III)$ ⁵, strongly suggestive of an evolutionary pressure preserving the pattern of the triple helical domain exons. The large number of introns inevitably raises questions about their possible function and origin as well as the role of the exons in the evolution of the protein molecules. Although it is clearly impossible to define 41 functional domains within the triple helical region, it is possible that this gene structure is a consequence of the existence of some functional domains, each encoded by a group of exons. For example, the charge distribution of these structural modules could be involved in the normal process of D-periodic fibre formation and possibly in the interactions of the collagens with other macromolecules¹. The observed conservation of exon size and arrangement may, therefore, indicate that the triple helical region of the fibrillar collagens is made up of different modular structural and/or functional units, hitherto masked by the repetitive nature of the (Gly-X-Y) units. For example, exons 7, 12, 15, 20 and 41 may represent such modular units. Exon 12 (amino acid residues 766–801) encodes the Gly-Ile vertebrate collagenase cleavage site as well as the putative fibronectin binding site amino acids (766–788)⁶. Both exon 7 (amino acids 910–945) and exon 41 (amino acids 73–90) contain the core structure Gly-X-Lys-Gly-His-Arg which has been implicated in interchain cross-linking with amino- and carboxy-terminal lysines when collagen is packed in a quarter-stagger arrangement^{7–9}. In addition, we have identified four other regions showing a related arrangement (Gly-X-Lys-Gly-X-Arg). One of these, amino acid residues 682–687, is contained in exon 15 (amino acids 676–693) and serves as a carbohydrate attachment site⁹. Another region, between amino acid residues 562 and 567, is present in exon 20 (amino acids 532–567) and is thought to interact with the carboxy-terminal allysyl or hydroxy-allysyl residue within the D stagger arrangement of collagen molecules⁹. The specific biological role, if any, of the other two related sequences, located in exon⁹ (amino acids 856–891) and exon 13 (amino acids 712–765), is not yet known.

As originally observed for the chick pro $\alpha 2(I)$ gene², the junctions between the central triple helical region and the terminal peptides are each located within single exons (4 and 46) coding for the propeptidase cleavage sites (Figs 2, 3). Similarly, the conserved size and distribution of the carboxy-terminal peptide exons strongly support the idea that they define functionally distinct subdomains² (Figs 2, 3). An identical exon-intron arrangement is also observed for the amino-terminal peptide of the pro $\alpha 1(I)$ (pN $\alpha 1(I)$) and pro $\alpha 2(I)$ ^{2,10} (pN $\alpha 2(I)$) collagens, although the coding capacity of the latter is 50% less than the former¹ (Figs 2–4). Exons 47, 49 and 51 represent

junction exons between the three regions which are conventionally recognized within the pN $\alpha 1(I)$ ¹ (Fig. 3). Interestingly, the amino-terminal globular region is clearly demarcated into three distinct subdomains with the 10 cystinyl residues involved in interchain disulphide bonding, clustered together in exon 50. In addition, the stretch of acidic amino acid residues in exon 51 seems to be particularly prone to changes compared with the mouse and chicken pN $\alpha 1(I)$ as well as the calf and chicken pN $\alpha 1(III)$ ^{11–14}. The last exon (51) of the human gene contains the signal peptidase cleavage site, which Tate *et al.*¹⁰ localized to the penultimate exon of the chicken pro $\alpha 2(I)$ gene. However, another potential signal peptidase site (Ala-Thr-Ser↓Gln) can be recognized in the last exon of the avian gene. On this basis and because of the structural analogies shared by the two genes, we favour the notion that the junction between the signal peptide and the amino-terminal domain is contained within the same exons, the last, in both genes. One implication is that the two very small exons (11 and 18 bp) of the avian gene are probably vestiges of larger coding units of the globular region, absent from pN $\alpha 2(I)$.

The structure of the collagen genes represents a unique example of a mosaic of functions which have been proposed for introns. The introns of the terminal propeptides seem to separate exons coding for functionally distinct domains, whereas the triple helical introns may stabilize the number of the repetitive coding units because of the rigorous length requirements needed for normal fibrillogenesis¹. Another alternative is that the triple helical exons represent different functional subdomains similar to those observed in the terminal propeptides. A third and more attractive possibility is that during evolution both phenomena have concurred in generating this complex gene structure which satisfies the structural and functional criteria mentioned above. In this context it is interesting that a 36-bp exon coding for four (Gly-X-Y) units is present in the less conserved amino-terminal propeptide region and that the exon structures of invertebrate collagen genes do not even remotely resemble the 54-bp coding unit².

Our results suggest that the type I collagen genes evolved on different chromosomes¹⁵ from a common multi-exon structure, which in turn may have originated from a basic 54-bp coding unit³, and diverge from each other both at the level of the coding sequences¹⁶ and of the size of the introns. The conservation of the exon structure indirectly indicates that a strong selective pressure has been exerted, probably because of rigorous length requirements of the protein molecules. Although gene families are considered to evolve in concert through gene conversion, genes on non-homologous chromosomes may differentiate more

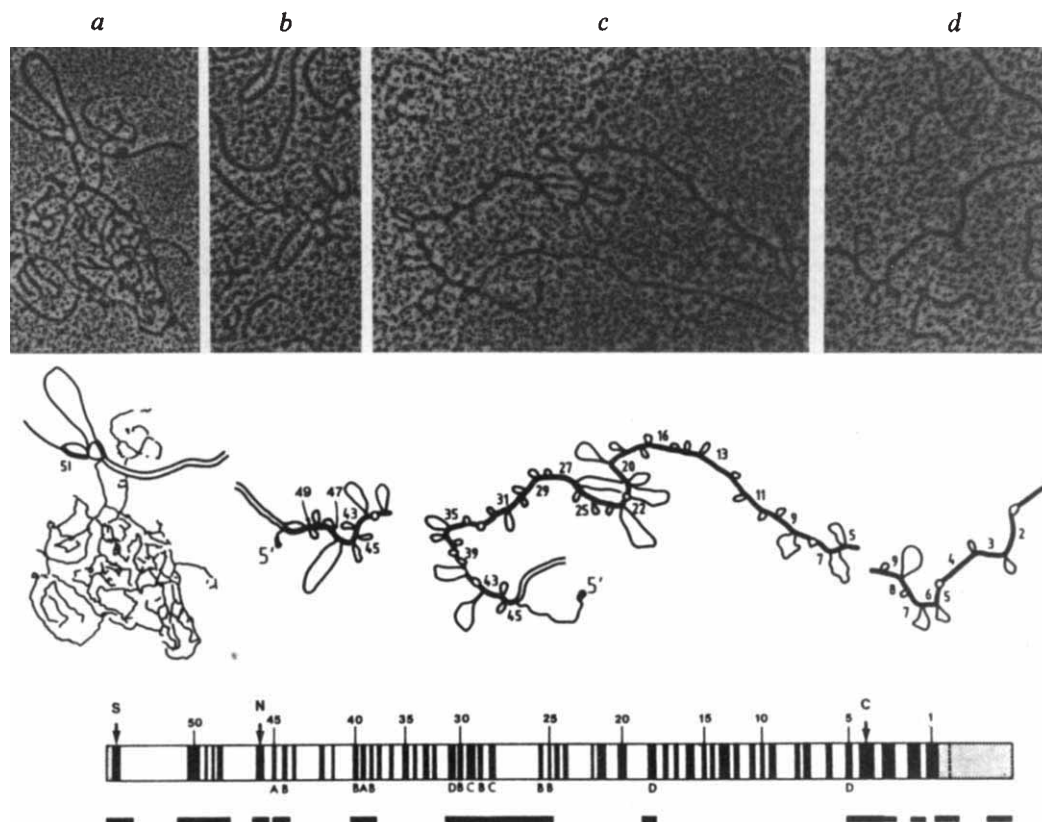


Fig. 2 Overall exon-intron structure of the *proα1(I)* collagen gene. R-loop analysis was carried out as described elsewhere¹⁸. DNA-RNA hybrids between *proα1(I)* mRNA and the RMS-8 4.8-kbp *Bam*HI subclone in pBR322 (*a*), the RMS-8 7.6-kbp *Hind*III-*Eco*RI subclone in pBR322 (*b*) and RMS-1 (*c* and *d*) were photographed with a Philips 301 electron microscope and digitized at a final magnification of $\times 150,000$. Single-stranded and the double-stranded replicative form of phage ϕ X174 were included for length calibration. Interpretive tracings are shown beneath the four micrographs. The numbers correspond to the exons, beginning at the 3' end of the gene. Parallel lines indicate vector sequences. The unhybridized 5' ends of *proα1(I)* mRNA are also shown in *b* and *c*. The diagram represents the schematic exon-intron map of the gene: black boxes correspond to exons, white boxes represent introns and grey boxes correspond to the 5' and 3' noncoding sequences, with the vertical dotted line indicating the end of the small polymorphic mRNA transcript (M.-L. Chu *et al.*, in preparation). The determination of the amino acid residues encoded by those exons which were not sequenced and which are discussed in the text, was based on hybridization analysis of various cDNA sub-fragments and on coding requirements. The black bars at the bottom of the figure indicate areas of the gene which were subjected to nucleotide sequencing; some of the sequences are shown in Fig. 4, the others either have been already reported¹⁶ or will be presented elsewhere (M.-L. Chu *et al.*, in preparation). The exact sizes of some of the triple-helical exons are indicated by the following: A, 45 bp; B, 54 bp; C, 99 bp; D, 108 bp. Note that the exon 13 is the largest exon of the triple helical domain⁶ (162 bp). The intron/exon ratios in the three regions coding for the different protein domains are 4 (N-terminal propeptide), 3 (triple helix) and 0.8 (C-terminal propeptide); these values are proportionally comparable with those of the *proα2(I)*^{2,18} and *proα1(III)*⁵ collagen genes, and similar to those of the *proα1(II)* gene (L. Sandell, personal communication).

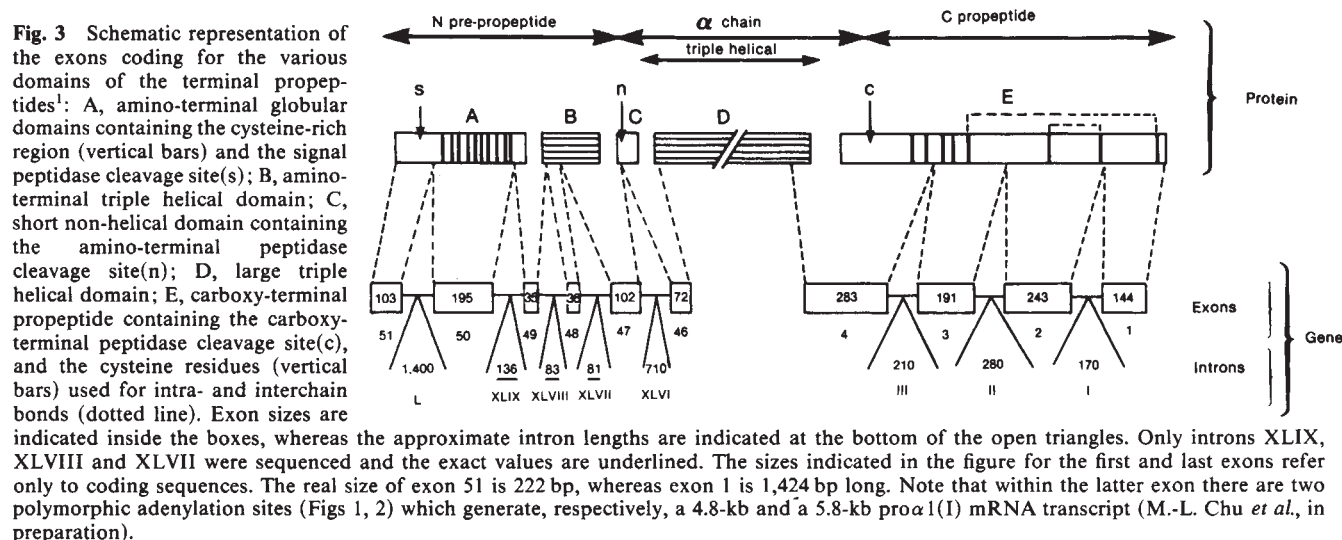


Fig. 3 Schematic representation of the exons coding for the various domains of the terminal propeptides: A, amino-terminal globular domains containing the cysteine-rich region (vertical bars) and the signal peptidase cleavage site(s); B, amino-terminal triple helical domain; C, short non-helical domain containing the amino-terminal peptidase cleavage site(n); D, large triple helical domain; E, carboxy-terminal propeptide containing the carboxy-terminal peptidase cleavage site(c), and the cysteine residues (vertical bars) used for intra- and interchain bonds (dotted line). Exon sizes are indicated inside the boxes, whereas the approximate intron lengths are indicated at the bottom of the open triangles. Only introns XLIX, XLVIII and XLVII were sequenced and the exact values are underlined. The sizes indicated in the figure for the first and last exons refer only to coding sequences. The real size of exon 51 is 222 bp, whereas exon 1 is 1,424 bp long. Note that within the latter exon there are two polymorphic adenylation sites (Figs 1, 2) which generate, respectively, a 4.8-kb and a 5.8-kb *proα1(I)* mRNA transcript (M.-L. Chu *et al.*, in preparation).

Human	ATG	TTC	AGC	TTT	GTG	GAC	CTC	CGG	CTC	CTG	CTC	CTC	TTA	GCG	GCC	ACC	GCC
Calf	Met	Phe	Ser	Phe	Val	Asp	Leu	Arg	Leu	Leu	Leu	Leu	Leu	Ala	Ala	Thr	Ala
Chick	*	*	*	*	*	*	Ser	*	*	*	*	*	Ile	*	*	*	Val
Mouse	*	*	*	*	*	*	*	*	*	*	*	*	*	Gly	*	*	*
CTC	CTG	ACG	CAC	GGC	CAA	GAG	GAA	GGC	CAA	GTC	GAG	GGC	CAA	GAC	GAA	GAC	ATC
Leu	Leu	Thr	His	Gly	Gln	Glu	Glu	Gly	Gln	Val	Glu	Gly	Gln	Asp	Glu	Asp	Ile
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*	*	*	Arg	*	*	Gly	*	*	*	*	*	*	*	*	*	*	Gln
CCA	ATC	ACC	TGC	GTA	CAG	AAC	GGC	CTC	AGG	TAC	CAT	GAC	CGA	GAC	GTG	TGG	AAA
Pro	Ile	Thr	Cys	Val	Gln	Asn	Gly	Leu	Arg	Tyr	His	Asp	Arg	Asp	Val	Trp	Lys
* Val	*	*	*	*	*	Asp	*	*	*	*	*	*	*	*	*	*	*
Thr	Gly	Ser	*	*	*	Asp	*	*	X	*	Asn	*	X	*	*	*	X
GAG	CCC	TGC	CAG	ATC	TGC	GTC	GAC	AAC	GGC	AAG	GTC	TTG	TGC	GAT	GAC	GTG	ATC
Glu	Pro	Cys	Gln	Ile	Cys	Val	Cys	Asn	Asn	Gly	Lys	Val	Leu	Cys	Asp	Asp	Ile
Val	*	*	*	*	*	*	*	*	*	*	Asn	*	*	*	*	*	*
TGT	GAC	GAG	ACC	AAG	AAC	TGC	CCC	GGC	GCC	GAA	GTC	CCC	GAG	GGC	GGC	TGC	TGT
Cys	Asp	Glu	Thr	Lys	Asn	Cys	Pro	Gly	Ala	Glu	Val	Pro	Glu	Gly	Glu	Cys	Cys
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GTC	TGC	CCC	GAC	GGC	TCA	GAG	TCA	CCC	ACC	GAC	CAA	ACC	ACC	GGC	GTC	GAG	GGA
Val	Cys	Pro	Asp	Gly	Ser	Glu	Ser	Pro	Thr	Asp	Gln	Glu	Thr	Gly	Val	Glu	Gly
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CCC	AAG	GGA	GAC	ACT	GGC	CCC	CGA	GGC	CCA	AGG	GGA	CCC	GCA	GGC	CCC	CCT	GGC
Pro	Lys	Gly	Asp	Thr	Gly	Pro	Arg	Gly	Pro	Arg	Gly	Pro	Ala	Gly	Pro	Pro	Arg
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GAT	GGC	ATC	CCT	GGA	CAG	CCT	GGA	CTT	CCC	GGA	CCC	CCC	GGA	CCC	CCC	GGA	CCT
Asp	Gly	Ile	Pro	Gly	Gln	Pro	Gly	Leu	Pro	Gly	Pro	Pro	Pro	Pro	Pro	Gly	Pro
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GGA	CCC	CCT	GGC	CTC	GGA	GGA	AA	TTT	GCT	CCC	CAG	CTG	TCT	TAT	GGC	TAT	GAT
Gly	Pro	Pro	Gly	Leu	Gly	Gly	Asn	Phe	Ala	Pro	Gln	Leu	Ser	Tyr	Gly	Tyr	Asp
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AAA	TCA	ACC	GGA	GGA	ATT	TCC	GTG	CCT	GGC	CCC	ATG						
Lys	Ser	Thr	Gly	Gly	Ile	Ser	Val	Pro	Gly	Pro	Met						
-	-	-	-	-	-	-	-	-	-	-	-						

Fig. 4 Nucleotide sequences of exons 46–51; the derived amino acid residues are compared with the corresponding sequences from calf, mouse and chicken^{11–14}. Asterisks represent identity with the human sequences; boxes identify deletions and dashes indicate that the sequences are unknown. The mouse amino acid sequences have been determined only for exon S1 (ref. 11). A high degree of nucleotide sequence conservation exists between the mouse and human gene from the beginning of intron L to position –215 (ref. 11 and M.-L. Chu *et al.*, in preparation). The amino acid residues are numbered from the initiation site of translation; the two arrows indicate the cleavage sites of the signal and amino-terminal peptidases. Triangles demarcate the exons. (Compare with the schematic representation in Fig. 3.) Only one of the five amino acid residues comprising the short non-helical stretch is coded for by exon 47, which also contains the sequences for 11 of the 16 (Gly-X-Y) units of the triple helical segment. The rest of the amino-terminal triple helical region is localized in exon 48 (4(Gly-X-Y) units) and in the 35-bp exon 49. The latter, in addition, encodes the last 8½ amino acids of the globular domain, thus serving as a junction between the globular and amino-terminal triple helical domains. Exon 50 (195 bp) codes for 65 amino acids of the globular domain, the remaining 12½ amino acids of which are encoded by exon 51. Exon 51 contains the sequences of the signal peptidase cleavage site, the signal peptide and the 119-bp-long 5'-untranslated region.

than those on homologous chromosomes¹⁷. It is tempting, therefore, to speculate that the observed differences in the length of the collagen genes may reflect the different chromosomal origin of two or more distinct gene clusters because of a faster rate of gene conversion within a given cluster or subfamily of genes.

We thank Dr T. Maniatis for his kind gift of the *AluI*–*HaeIII* human genomic library; Drs H. Boedtker, B. de Crombrughe, R. Jaenisch, L. Sandell, W. Upholt and Y. Yamada for communicating results prior to publication; Drs A. Bank, D. Broeck, B. R. Olsen and D. J. Prockop for helpful suggestions; and Ms K. Valentine for typing the manuscript. W. de W. thanks Drs

Jan Coetzee and Chris van der Merwe (Department of Electromicroscopy, University of Pretoria) for providing facilities. This work was supported by grants from NIH (AM 32380), the March of Dimes-Birth Defects Foundation, and the South African Council for Scientific and Industrial Research (CSP Biotechnology Programme).

Received 23 February; accepted 30 May 1984.

- Bornstein, P. & Sage, H. A. *Rev. Biochem.* **49**, 957–1003 (1980).
- Tate, V., Finer, M., Boedtker, H. & Doty, P. *Cold Spring Harb. Symp. quant. Biol.* **47**, 1039–1049 (1982).
- Yamada, Y. *et al. Cell* **22**, 887–892.
- Sandell, L. *et al. J. biol. Chem.* **258**, 11617–11621 (1982).
- Yamada, Y. *et al. J. biol. Chem.* **258**, 2758–2761 (1983).
- Monson, J. M. *et al. Molec. cell. Biol.* **2**, 1362–1371 (1982).
- Fietzek, P. P. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 84–86 (1977).
- Piez, K. A. in *Biochemistry of Collagen* (eds Ramachandran, G. N. & Reddi, A. H.) 1–84 (Plenum, New York, 1976).
- Fietzek, P. P. & Kuhn, K. *Int. Rev. Connective Tissue Res.* **7**, 1–60 (1976).
- Tate, V. *et al. Nucleic Acids Res.* **11**, 91–103 (1983).
- Harbers, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 1504–1508 (1984).
- Pesciotta, E. M. *et al. Biochemistry* **19**, 2447–2454 (1980).
- Yamada, Y. *et al. J. biol. Chem.* **258**, 14914–14919 (1983).
- Timpl, R. & Glanville, R. W. *Clin. Orthop. Related Res.* **158**, 224–242 (1981).
- Huerre, C. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6627–6630 (1982).
- Bernard, M. P. *et al. Biochemistry* **22**, 5213–5223 (1983).
- Ohta, T. & Dover, G. A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4079–4083 (1983).
- Myers, J. C. *et al. J. biol. Chem.* **258**, 10128–10135 (1983).
- Chu, M.-L. *et al. Nucleic Acids Res.* **10**, 5424–5434 (1982).

Errata

Holocene alluviation and hydrology in the upper Thames basin

M. A. Robinson & G. H. Lambrick
Nature **308**, 809–814 (1984)

ON page 811, Fig. 2, site 1, ~AD 7 should read 7th century AD and, similarly, mid AD 10 in Fig. 3, site 8 should be mid-10th century AD.

Auditory receptive fields in primate superior colliculus shift with changes in eye position

Martha F. Jay & David L. Sparks
Nature **309**, 345–347 (1984)

THE following lines of text were omitted from page 347, second paragraph, between 'With gaze directed at the centre fixation' and 'SR burst cells tested in this manner':

'...light, saccades to an auditory or visual target placed 10° above and 16° to the left of centre were preceded by a vigorous burst of activity. When the leftward initial fixation point was viewed, requiring a saccade to this same target of 10° up and 8° right, the neurone failed to produce a burst of activity regardless of target modality. Comparable results were obtained from the 23 ...'.

Isotope studies of insular phosphates explain atoll phosphatization

Paul Aharon & H. H. Veeh
Nature **309**, 614–617 (1984)

ON page 614, in the second sentence of the first paragraph, 'pohsphates' should read 'phosphates'. In Table 1, the second entry under 'Nauru' should be NRU-2, not NRU-5, and in the last line of the table legend, the 1σ error is incorrectly given as ±1%—it should read ±0.1%.

Improving on Newton

Oliver Penrose

Order out of Chaos: Man's New Dialogue with Nature.

By Ilya Prigogine and Isabelle Stengers.

Bantam, New York: 1984. Pp.349. Pbk \$8.95.

To be published in the UK on 6 August by William Heinemann, hbk £9.95.

WHEN Galileo and Newton formulated the laws of classical mechanics, they revolutionized man's understanding of the Universe and his power to predict and control the behaviour of small parts of it. Nevertheless two aspects of Newtonian mechanics have puzzled people who looked at the world about them and wondered whether Newtonian mechanics was sufficient to explain its behaviour. One of these aspects is its determinism — the fact that the laws of motion, taken together with the dynamical state of the Universe (the positions and the velocities of all the particles in it) at some arbitrary instant, completely determine the entire past and future of the Universe. This is, to say the least, somewhat humiliating to us human beings who do not like to think that the actions and decisions we agonize over, the immortal flashes of creative inspiration, have "in reality" been predetermined since the world began. Somehow, it seems, human beings have been left out of the picture. In the words of Jacques Monod, cited on page 3 of the book under review:

Man must... at last realize that, like a gypsy, he lives on the boundary of an alien world. A world that is deaf to his music, just as indifferent to his hopes as it is to his suffering or his crimes.

The other puzzling aspect of Newtonian mechanics is its symmetry under time reversal: for every possible motion of a dynamical system there is another in which the same sequence of dynamical states is traced out in precisely the opposite order. The behaviour of real physical systems, on the other hand, is rarely symmetrical under time reversal; and so Newtonian mechanics alone is not sufficient to explain their behaviour.

The twentieth century has seen two further revolutions in the science of mechanics, but neither has totally overcome the misgivings aroused by Newtonian mechanics. Relativity theory, with its emphasis on the role of the observer, seems at first sight to bring human beings into the picture; but the resulting picture is no less deterministic than Newton's. Indeed Einstein himself, on the occasion of the death of his close friend, Michele Besso, wrote:

Michele has left this strange world just before me. This is of no importance. For us convinced physicists the distinction between past, present and future is an illusion, although a persistent one.

Underlying this poignant passage (another

of the many interesting quotations in the book under review) can be discerned the determinist's belief that past, present and future are logically equivalent: any one of them determines the other two. (Though not every determinist would accept Einstein's inference that the distinction between past, present and future is therefore illusory). Likewise, relativistic mechanics offers us no more than Newton in the way of an explanation of time asymmetry: its laws are just as symmetrical as those of Newton.

Quantum mechanics was a more far-reaching revolution; like relativity, it stresses the role of the observer, but, unlike relativity, it also stresses the uncontrollable and non-deterministic character of his interaction with the system he observes. Nevertheless, the puzzlement remains. The time evolution of an unobserved system is still governed by a deterministic law — Schrödinger's equation — and so if we choose to regard the entire Universe as a single system there is no outside observer to interfere uncontrollably, and the determinism is still there. Quantum mechanics also fails to remove our puzzlement about time asymmetry. Schrodinger's equation has a time-reversal symmetry similar to that of Newton's equations; so the only answer quantum mechanics has to offer to the time-asymmetry puzzle is that we as observers may be inserting the time asymmetry into the systems we study. It is hard to accept this explanation since one believes that there are irreversible processes taking place in systems such as the centres of stars which have never been directly observed and never will be.

In their new book *Order out of Chaos* (in effect a new edition of their earlier *La Nouvelle Alliance*, published by Gallimard in 1979), Isabelle Stengers and Ilya Prigogine make a new assault on these well-entrenched perplexities. Their thesis is that a further revolution in science is now taking place in which the sterile view of the world provided by mechanics is being replaced by a more complicated and fruitful one; but this time the revolution consists not in yet another advance in the science of mechanics but in an increased emphasis on irreversible (time-asymmetric) aspects of nature.

The book is in three main parts. Part I is a scholarly examination of the Newtonian world view and of the comments which a large array of philosophers and other savants have made about it over three

centuries. For me the most interesting section in this part of the book was the one dealing with the views of the anti-scientific writers such as Heidegger and Koestler. Part II is devoted to phenomena which seem outside the Newtonian view because they are unpredictable or time-asymmetric or both; it includes examples from a variety of branches of science where irreversible processes, taking place far from equilibrium, do indeed generate "order out of chaos" rather than the degradation of order into chaos which the second law of thermodynamics would lead one to expect. In particular, it is here that biological systems make their first appearance in the book.

The final part investigates what post-Newtonian mechanics, particularly quantum mechanics and statistical mechanics, can do to improve on the Newtonian picture. It includes a non-technical description of Prigogine's own work in this area. The motivation for this work appears to be a dissatisfaction with the "Newtonian" features of the conventional formalism of statistical mechanics as developed by its founding father, Willard Gibbs. The Gibbs formalism, based on time-dependent probability distributions in phase space, is symmetrical under time reversal; it contains no criterion for distinguishing a physically possible non-equilibrium probability distribution from the physically impossible one that would be obtained from it by time reversal. Despite many attempts, both by Prigogine and others, for "breaking the time-reversal symmetry" (that is, for inserting unsymmetric elements into the theory which would enable us to distinguish the physical from the non-physical probability distributions), I believe that the definitive answer to this problem has not been given.

Some readers may be irritated by the rather effusive style, which in places reads almost like advertising copy ("It is hardly an exaggeration to state that one of the greatest dates in the history of mankind was April 28, 1686, when Newton presented his *Principia* to the Royal Society of London") or by some minor imperfections in the English (the word "modelization", for example, is not part of the language as I know it). More serious, perhaps, are a certain lack of logical focus in the writing and a few inaccuracies of fact (one is in the sentence cited above; only Part I of *Principia* was presented on April 28, 1686, Parts II and III appearing later on). In a mathematical passage even such a small inaccuracy can easily make the text almost unintelligible. But the book contains plenty of interesting information and has much to offer readers with interdisciplinary interests in physics, chemistry, biology or the history and philosophy of science. □

Oliver Penrose is Professor of Mathematics at the Open University.

Engineering stories of success

Donald S.L. Cardwell

From Compass to Computer.

By W.A. Atherton.

Macmillan Press, London/San Francisco Press, Box 6800, San Francisco, CA 94101: 1984. Pp. 337. Hbk £20, \$30; pbk £9.95.

DR ATHERTON'S account of the rise of electrical and electronic engineering is the first attempt at a general history since Dr Dunsheath's *A History of Electrical Engineering* was published over 20 years ago. It is an ambitious undertaking. Following an introductory chapter dealing with progress before 1800, there are chapters on the electric telegraph, the experimental and theoretical advances of the nineteenth century, electric power, electric lighting, radio, miniaturization and the computer.

In the nature of the case Dr Atherton has had to compress so much and cut so many corners that, at times, his narrative comes close to being a breathless recital of names and inventions. Attempts to cope with the complexity of the subject matter have led to other problems. Thus, having described the development of Maxwell's theory up to relativity and quantum theory, the author gives his readers a cultural jolt by taking them back to the invention of the electric telegraph in the early nineteenth century. There is also some, probably unavoidable, repetition: Davy's arc light, Schweigger's "multiplier", the Gramme dynamo are described twice. In fact, so complicated is the story that what we are given is a series of autonomous essays on key historical developments. If the book is read in such fashion, these criticisms lose some of their force.

Dr Atherton explains that he is writing for the engineer and the general reader, not for the historian. Fair enough; but more attention to the historical record would have saved him from some mistakes and some omissions. For example, it was not Faraday but Whewell who coined the words "anode", "ion", etc; Joule's papers on the mechanical equivalent of heat were *not* denied publication by scientific journals; lamination, to reduce eddy currents, was introduced in the 1830s, not in 1870. Had the author used standard sources, such as the *Dictionary of Scientific Biography*, he would have avoided these and some other slips.

On the other hand Dr Atherton does not mention what I have called the "electrical euphoria" that swept Europe and the United States in the late 1830s. It was widely, and plausibly, believed that the electric motor, driven by voltaic cells, could be made more efficient than the best steam engine. This was not the last time that there were great hopes of cheap electric power.

The next occasion — also not mentioned by Dr Atherton — was just over 40 years later.

In this context, the observant reader may be puzzled by the *Punch* cartoon of 1881, reproduced on page 148 of the book and also here. It shows King Steam and King Coal anxiously contemplating a baby in a cradle. The baby has the word "electricity" over his head and is holding a large feeding bottle with "Storage of Force" written on it. Enigmatic though these three words are, the reader is left to infer that the cartoonist foresaw the modern electrical supply industry. This would be quite wrong. In 1881 Faure's improved secondary cell, or rechargeable storage battery, caused great excitement. It was greeted as the invention that would bring about the hoped-for revolution in electricity. It offered complete flexibility, for every little stream, every hill-top open to the winds meant an opportunity to charge and recharge storage batteries that could be used for all practical purposes. But the way ahead was that followed by Edison and Ferranti; and the forecasts of 1881 have not been realized, beyond today's "alternative technology". In short, this work has little to say about false starts and misplaced hopes. It is a success story; a thoroughgoing Whig interpretation of the history of technology.

Dr Atherton writes clearly and wittily



"WHAT WILL HE GROW TO?"

and his book, apart from one or two unsatisfactory diagrams, is well produced. It should interest electrical engineers and in that respect it will serve its purpose. But a rather more modest programme and a little more care with historical material would have meant a better book. □

Donald S.L. Cardwell is Professor in and Head of the Department of History of Science and Technology at UMIST, Manchester.

Power and power

Eli B. Roth

The State and Nuclear Power: Conflict and Control in the Western World.

By J.A. Camilleri.

Harvester Press/University of Washington Press: 1984. Pp. 347. £25, \$25.

IN *The State and Nuclear Power*, Professor Camilleri's thesis is that the pro- and anti-nuclear elements in society present a new destabilizing and potentially fatal challenge to the State. By "the State" he means "capitalist" countries, taking the United States, France, Britain, West Germany, Sweden and Brazil as specific cases. He explicitly excludes from his analysis centrally-planned, "non-capitalist" economies, otherwise known as authoritarian states. The reason, he says, is brevity, but one might suspect a more important reason to be the difficulty in getting dependable data from such nations.

Camilleri notes of the countries he has chosen for discussion:

The actual strategies adopted by each state in response to the ethical challenge posed by anti-nuclear activism varied considerably from country to country. A critical factor in all cases was the distribution of power in the political system, that is, the degree of centralisation in the exercise of state authority. As a broad generalisation, it would appear that where the state apparatus operated as a cohesive, well-

coordinated, monolith entity faithfully reflecting the interests of a dominant power group, its actions tended to dismiss the relevance of normative considerations and to reinforce the supremacy of technical rationality [p.115].

This tendency to criticize or ignore technical problems and solutions is one of the shortcomings of the book, and may also explain why Camilleri omits Canada from his list of case studies. Canada is as much of a capitalist country as any of the other nations he does examine. But while Canada generated in 1983 a larger percentage of its electricity from nuclear power plants than the United States, Britain or West Germany, it has suffered no noticeable internal political strains because of it. The most obvious distinction between Canada and the others lies in the type of reactor programme followed, one based on the home-grown CANDU plant which is fundamentally different from the power plants used by his chosen examples.

The book raises some crucial questions about the impact of possibly faulted, ill-conceived or incomplete nuclear programmes on democratically-run governments. Camilleri asks, "What factors account for the commitment of major western governments to commercial developments? . . . To what extent, and in what respect, has the performance of these [economic and administrative] functions varied from state to state? How may those differences be explained?". The questions are especially well-formulated in his preface, while the case-histories which

follow are clear and well researched. Of special value is the brief chapter dealing with elements of uranium supply and demand, enrichment, reprocessing and nuclear waste. Here Camilleri juxtaposes with considerable clarity the history of the growth of nuclear power programmes in each of his six chosen countries; together with some of the other material in the book, this account will be a valuable reference source.

In contrast, the treatment of international nuclear politics touching on non-proliferation matters is sketchy, and at times misses the mark, mixing cause with effect or ascribing the wrong motives to policies. For example after going into substantial detail (pp.253–267) to describe the actions taken by the United States in support of the non-proliferation regime under President Carter's leadership, Camilleri dismisses the performance as "a situation from which US nuclear export policy sought to derive maximum advantage [p.288]". This evaluation runs contrary to most informed judgements that the Carter policy was, in fact, a colossal misapplication of means to ends — although with commendable motives — in support of the NPT. In fact, the United States lost nuclear-power-related business in international markets to other supplier countries which could furnish the technology and which got the orders as a direct result of Carter's policies.

Because Camilleri explicitly excludes from his book the nuclear power programmes behind the Iron Curtain, we do not get the full benefit of his considerable talents at digging out and organizing the events of recent history. Had he done so, I wonder whether he would not have stressed the West's *democratic* institutions instead of focusing on their *capitalistic* economies as the root cause of the troubles with their nuclear programmes — to my knowledge, there is no case where popular objections have been reported as having interfered with the prosecution of the nuclear programmes in the states with "centrally-planned economies".

This is a substantive and provocative book which deserves a wide audience. As an academic and an economist, however, Camilleri seems to be writing exclusively for his peers, while his ponderous style with its overload of jargon demands a high degree of perseverance on the part of the reader. Equally, a shoddy publishing job — including proof-reading errors and uneven, over-compressed typography — has made the book uncomfortable going. But none of these points should be allowed to detract from Camilleri's scholarly analysis of some of the most pressing problems now facing those democratic nations pursuing nuclear power programmes. □

Eli B. Roth is a consultant on energy technology and policy, and a Visiting Fellow at Yale University.

Growth of an infant science

S.D. Wratten

Insects on Plants: Community Patterns and Mechanisms.

By D.R. Strong, J.H. Lawton and Sir Richard Southwood.

Blackwell Scientific/Harvard University Press: 1984. Pp.313. Hbk £22, \$35; pbk £11.80, \$18.

ON THE last page of this book, the ecology of insect-plant interactions is dubbed "our infant science". The phrase is a good guide to the excitement and frustrations of working in this research area in the 1980s. The excitement, which the book certainly reflects, comes from the frequency with which a few empirical but well-conducted experiments have produced far-reaching ideas demanding further testing. The frustrations are the familiar ones of having insufficient time and manpower to test the hypotheses which are produced by a burgeoning discipline.

I read the book on an otherwise tedious journey on a slow Spanish train and my attention was held throughout. However, it is the sort of book that also demands that the reader put it down at frequent intervals, simply because, like all good textbooks, it asks questions. Those it asks most penetratingly concern the frequency and importance of competition between plant-feeding insects and the role of coevolution as a driving force in plant-herbivore relationships. Having posed the questions, the authors do not shrink from attempting to answer them, sometimes with a conviction which belies the tender age of the subject.

One of the current debates is over how often plant resources are limited to such an extent that insect species compete for them. The authors consider this inter-specific competition to be a rarity, and are more impressed with the apparent evidence that predators and parasitoids keep insect numbers below levels at which competition occurs. They review published life-table analyses of populations of phytophagous insects and show that even cases of intra-specific competition (a necessary precursor of inter-specific competition) are outnumbered 2:1 by those of predation as factors acting in a density-dependent way, that is factors which increase the proportional mortality they impose as populations increase. Support for the premise that plant-feeding insect species rarely compete is taken also from the "stunningly simple logic" of Hairston *et al.* (*Am. Nat.* 44, 421–425; 1960) who argued that

obvious depletions of green plants by herbivores are exceptions to the general picture that green plants are abundant and largely intact. The only possible remaining general method of control is

predation ... including parasitism. Herbivores are ... therefore not likely to compete for common resources.

What is intuitively logical is not always correct, however, as the statistician G.E. Yule implied when he wrote that "Logic and Mathematics are only of service ... once you have found the right track". Another review of a different set of population analyses has indicated that there is, indeed, a different track which could be followed. J.P. Dempster (*Biol. Rev.* 58, 461–481; 1983) surveyed 24 studies of moth and butterfly populations and concluded that intra-specific competition was the only density-dependence operating in 13 cases, with natural enemies exhibiting density dependence in another three. These cases of intra-specific competition usually involved clear depletion of food resources but recent work (much of which has appeared since this book was written) has added extra evidence that apparent super-abundance of acceptable food may not be the norm.

This topic, given only half a page in the book, concerns the role of feeding-induced changes in plants following insect feeding. If such "induced defences" are common they could lead to an increasing proportion of a plant's leaves becoming unsuitable as grazing by an insect population progresses, forcing competition upon individuals when crude measures of foliage biomass would still indicate an apparent super-abundance. C. West, for example, in a paper to be published (*Ecol. Ent.* 10; 1985), shows that oak leaf miner larval fitness is reduced if the larvae feed on leaves which have earlier sustained normal levels of caterpillar damage. These results point to temporal competitive exclusion between the mining and chewing guilds; if they represent hitherto under-investigated interactions, they cast doubt on the premise that inter-specific competition among phytophages is a rarity.

The ecological importance and topicality of these questions confirm that the time is certainly right for this book. The fact that the authors take definite stances on controversial areas make it stimulating reading. Final-year undergraduates and postgraduates are bound to enthuse about the clarity of writing and the layout, the attractive use of vignettes in the figures and — above all — about the excitement which this book conveys about "our infant science" and its future. □

S.D. Wratten is a Lecturer in the Department of Biology, University of Southampton.

Biotechnology in America

Edward Yoxen's *The Gene Business: Who Should Control Biotechnology?*, an examination of the environmental, industrial and social ramifications of genetic engineering, has been published in the United States by Harper & Row. Price is \$15.95. The original edition of the book, a paperback published in Britain by Pan (£3.95), was reviewed in *Nature* 304, 285; 1983.

Everything in the garden . . .

Peter D. Moore

The European Garden Flora. Vol.II Monocotyledons (Part II).

Edited by S.M. Walters *et al.*
Cambridge University Press: 1984.
Pp.318. £30, \$59.50.

IT MAY seem unlikely, but gardens can be frustrating places for the botanist. The problem is one of taxonomy. Out in the field, things are much easier; whether on the Downs of Sussex or in the swamps of the Nile Delta, there is sure to be a local *Flora* which permits the identification of just about any plant that you come across. But in the garden the troubles really start, for that tantalizing *Saxifraga* could have come from almost anywhere, from the Himalayas to Alaska, and where is the *Flora* which can hope to cover that span? The answer, it seems, lies in *The European Garden Flora*.

Sponsored by the Royal Horticultural Society and steered by an editorial committee of eminent botanists and horticulturalists, the series seeks to cover all the plants likely to be found in cultivation in Europe, whether in the open or under glass. The only plants deliberately omitted are the crop species and, of course, the weeds.

Such an extraordinarily ambitious project is made no easier by the confused state of horticultural taxonomy and nomenclature: indeed, if the series serves only to untangle some of the classificatory knots and proliferating synonyms it will have achieved a great deal. Add to these problems morphological variations which have resulted from generations of selective breeding in some taxa, and one cannot but admire the vision and determination of those who planned the work.

Volume II (of a total of six) completes the coverage of the Monocotyledons (begun in Vol.I, to be published later this year), and over half of it is devoted to the orchids. For each family a dichotomous key to genera is provided, and a key to families is given at the front of the volume. All keys are arranged in a concise yet clear form, with alternatives placed in a consecutive manner. Technical terms are kept to a minimum and a glossary is provided, as the book is intended to be used by gardeners as well as by taxonomists. This aim is evident also from the text, for the description of each species includes details of where a good illustration can be found, what variants are available in cultivation, and how the species is best grown in Europe. But Europe is a big place and contains a variety of climatic regions which obviously influence what plants can be grown. To overcome the problem, a hardiness code has been devised, based on the mean

January isotherms, and a map of Europe is provided showing their positions. This feature should prove a valuable aid in the selection of species for planting.

A book such as this is not, perhaps, the place to seek personal preferences and opinions from the authors. There are, however, some telling comments, such as "the unsightly variegated form of *Phalaris arundinacea* is a depressing feature of many an ill-tended border" — a refreshing reminder that both taxonomists and gardeners are human.

There is a wealth of information here, both scientific and practical. The authors have achieved what might have seemed impossible; they have taken a step towards the relief of the botanist's frustration in the garden and at the same time they have provided the horticulturalist with a concise compendium of modern botanical knowledge. □

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences, King's College, University of London.

Pictures in mind

Stuart Sutherland

Ghosts in the Mind's Machine: Creating and Using Images in the Brain.

By Stephen Michael Kosslyn.
W.W. Norton: 1984. Pp.249. \$19.95, £15.95.

WHEN asked whether a horse's tail ends above its knee, most people generate a visual image in thinking of the answer. Scientists, artists, engineers and writers constantly use images in their work. Hopes, fears and fantasies are often expressed in images and we cannot escape them even in sleep. Yet until recently there has been little systematic investigation of imagery.

Professor S.M. Kosslyn has put this right by conducting a series of fascinating experiments and proposing a most ingenious theory. The experiments could have been run a hundred years ago, since they do not require any apparatus more elaborate than a few drawings and a timing device. The theory, however, could not have been developed until recently since working out its details required computer simulation. The computer model certainly helped Kosslyn to ask the right questions: the work of his predecessors in the field, such as Francis Galton, is pallid by comparison, possibly because they had no way of formulating a precise theory of imagery that would direct their research. Kosslyn's agreeable book, *Ghosts in the Mind's Machine*, makes his work accessible to the general reader: it is clearly and simply written, and dispenses with jargon.

One of Kosslyn's more remarkable results is that mental imagery obeys many of the same laws as perception, even when

these laws are unknown to the subject. In normally sighted people, acuity for oblique striations is worse than for vertical or horizontal ones. Kosslyn asked subjects to image stripes of a given width at different orientations and to imagine themselves walking backwards away from the stripes: they had to give the distance at which the stripes became so blurred that they could no longer be distinguished. This distance was the same for subjects imaging the stripes as for those actually seeing them and for both groups the oblique stripes blurred at a shorter distance than the horizontal or vertical. Although Kosslyn does not say so, this finding and others he has obtained suggest that the neurophysiological medium responsible for imagery is the same as that used in vision. The low acuity for oblique stripes is thought to be caused by central mechanisms: it would be interesting to discover whether visual phenomena that were purely due to retinal interactions failed to occur in imagery.

Among Kosslyn's other findings are the following. The extent of a visual image is about the same as that of the visual field, and the amount of detail that can be represented declines rapidly from the centre to the outer parts of the image. Subjects can "scan" across an image and the further they have to scan the longer it takes. They can also perform a number of other transformations on the image or on a part of it, including zooming, panning and rotating. He produces evidence to show that an image is built up piece-meal out of separate components — in imaging a person, a broad outline is first constructed and then the details of the face, arms, legs and so on are added.

In his theory, Kosslyn posits that in long-term memory pictorial data is held separately from other forms of knowledge, though the two can interact. In imaging, some of the pictorial data is reproduced in a medium of limited extent which is fine grained in the centre and coarse grained in its outer parts. A procedure called PICTURE recovers the required pictorial data from long-term memory and places it in this medium, starting with a skeleton of the required image and filling in the details of its parts by the use of subsidiary procedures. One of these, named FIND, locates the correct point in the image at which to insert a more detailed representation of its parts: FIND operates by a random search, which seems a little implausible. The same procedure can be used to find a part of an image when information about it is needed, as for example to respond to the question "Do frogs have a tail?". Further procedures, such as SCAN, PAN and ROTATE, apply transforms to the image.

The results of applying the various operations are shown on a VDU, but although this is a good way of displaying them, Kosslyn is careful to point out that the processes underlying images do not have to be laid out in a pictorial space in the

brain. A computer can have a functional matrix of points stored at different addresses: the matrix does not need to be systematically arranged in space. Provided information is stored with each address to indicate its pictorial relation to other points, the information stored at the different addresses can be treated by the central processor as a picture. Kosslyn believes that the brain might function in a similar way. He puts his argument well and it should give pause to anyone who still believes that the operations that can be performed on visual images support the doctrine of isomorphism (that is the idea that each phenomenological dimension is represented by a corresponding physical dimension in the brain). It should, however, be noted that visual images could equally well depend upon the topological maps that are present in the primary visual areas: if so, they would involve some degree of isomorphism.

Kosslyn also rebuts those critics who have maintained that his results are produced entirely through subjects' expectations or by the experimental demands made on them. His experiment on stripes is enough to do this on its own, but for good measure he adds several others. For example, it has been argued that the rotation of mental images is a result of experience with objects rotating in the real world and that the speed at which a mental image is rotated depends on the subjects' expectations. Kosslyn shows that when subjects are required to rotate visual images of two different objects on a wheel, the speed of image rotation does not depend on the weight of the objects. Nevertheless, the subjects knew that in the physical world inertia would cause the heavier one at first to rotate more slowly than the lighter one, and could take this knowledge into account if required.

The least convincing part of Kosslyn's book is that dealing with the practical applications of his work. He suggests that it might be possible to discover which of the many routines he incorporates into his own program are poor in children who have deficient imagery: one could then concentrate on putting these routines right. More imaginatively, he proposes that the hallucinations experienced by schizophrenics are caused by deficiencies in the imaging process and might be corrected in the same way. The ideas are interesting but speculative: moreover, there is no evidence that people who claim to have no visual imagery suffer any intellectual loss. Nevertheless, I liked Kosslyn's tale of Nikola Tesla, the inventor of the AC generator, who claimed that having designed a machine he could run it in imagery for several weeks and then examine its parts to see how well they were wearing. It is a mercy he never applied his gifts to designing aero-engines. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition, University of Sussex.

Another tool for the biochemist

Masamichi Tsuboi

Applications of Infrared, Raman, and Resonance Raman Spectroscopy in Biochemistry.

By Frank S. Parker.

Plenum: 1983. Pp.550. \$65, £50.05.

VIBRATIONAL spectroscopy (infrared and Raman spectroscopy) is not used by all biochemists. It is of little help, for example, in locating and measuring a newly found protein with a special biological activity. However, when the molecular conformation or conformational change of the new actor becomes the subject for study, vibrational spectroscopy is a powerful tool. For such tasks its use is extending steadily; we may dream that one day vibrational spectra of macromolecules will become the objects of routine analysis through a mathematical treatment, like X-ray diffractions or NMR spectra, after which we shall acquire a firm knowledge of the molecular force field with the assistance of *ab initio* molecular-orbital calculations. At present, however, the analysis is essentially empirical and requires the accumulation of great numbers of reference spectra. It also demands skill and experience on the part of the practitioners, a fact which seems to cause them no little pleasure.

In his new book, Frank S. Parker nicely surveys the present state of vibrational spectroscopy as applied to studies of biological macromolecules. The book

opens with an attractive exposition of Fourier transform IR and other spectroscopic techniques and sampling methods. Dr Parker then deals with the applications of spectroscopy in a chapter on polypeptides and proteins, following this with descriptions of the spectra of eye tissues, purple membrane, enzymes, haemoproteins, metalloproteins, carbohydrates, nucleic acids and so on. Special emphasis is placed on the recent development of resonance Raman spectroscopy, which has brought great improvements in sensitivity and selectivity. Finally, Dr Parker covers the spectroscopic analysis of viruses and of biomembranes.

While the book is a review volume, covering much of the recent literature, it is written in a relaxed style which makes it much more pleasant reading than most of the papers on which it draws. The large number of illustrations — 322 of them — and the tables add a great deal further to the digestibility of the material. In organization, though, this is not a textbook. There is some repetition and choice of subject matter according to the author's preference; nevertheless graduate students in biology and chemistry will find it most instructive. Nor is it a handbook. For example the reference list at the end of each chapter is not intended to be exhaustive; but the information is well sign-posted and easily accessible. Above all, the work is permeated with Dr Parker's evident enthusiasm for the subject which makes his book unusually enjoyable. □

Masamichi Tsuboi is a Professor of Physical Chemistry at the Faculty of Pharmaceutical Sciences, University of Tokyo.



Bat's eye view — the photograph, of Epauletted Fruit bats (*Epomophorus anurus*), is reproduced upside down from Wilfried Schober's *The Lives of Bats*. The book is a translation and revision of the original German edition and is published by Croom Helm in Britain and Arco in the United States. It will be reviewed in a future issue of *Nature*, together with *Bats: A Natural History* by John E. Hill and James D. Smith (British Museum (Natural History)/University of Texas Press).

E. Kulzer.

Taxonomy across the language barrier

C.A. Stace

Grasses of the Soviet Union, in two volumes.

By N.N. Tsvelev. Translated by B.R. Sharma.

Oxonian Press, New Delhi (distributed by A.A. Balkema, PO Box 1675, Rotterdam, Netherlands): 1984. Pp.1, 196. Dfl.145, \$50, £34.

THOSE who doubt that language is still one of the world's greatest barriers should scan the references in a range of scientific papers written in different languages, when the bias towards the mother tongue will soon become obvious. While English-speakers are often able to get the gist of papers written in most European languages, the huge fund of scientific information written in Russian and Japanese (*inter alia*) is out of the reach of the vast majority of Western scientists.

The programme of translations of scientific works in these two languages (and others) being carried out by the Oxonian Press in India is therefore a notable service to the academic community. About 200 books have been translated, mostly in the past ten years and mostly from Russian, on disciplines ranging widely in the sciences

but with an emphasis on biology and on agriculture. The work reviewed here is a translation of N.N. Tsvelev's *Poaceae U.R.S.S.* of 1976, itself effectively an updating of the classic second volume (Gramineae) of *Flora U.R.S.S.* published in 1934.

Grasses are, without doubt, the most important group of organisms as far as mankind is concerned. The development of cereals can be said to have constituted the birth of agriculture and to have signalled the development of civilization, and today grasses are still the staple food of most races of man and of the animals he keeps for food. Our continued welfare depends upon improved methods of grass utilization, including the exploitation of new kinds of grass. A detailed knowledge of the grass flora of the USSR, said to number 1,011 species (compared with 880 in Europe, including European Russia), is therefore vital.

The first 67 pages of the present work are taken up with an essay on the structure and supposed evolutionary trends of grasses, followed by a map and detailed list of the phytogeographical divisions of the USSR. The most important part of Tsvelev's monograph is, however, the systematic treatment, which occupies 1,004 pages. In it, a key to genera and keys to species and subspecies within each genus are provided, as is a detailed classification of subfamilies, tribes, subtribes and various infra-generic ranks. Each of the taxa at the ranks from subfamily to generic section is provided with an extended diagnosis, but there are no descriptions of species or lower taxa — for these one has to rely on the information in the keys. Under each species there are full nomenclatural and distributional details, chromosome numbers (including some previously unpublished counts), and frequently a valuable discussion of unusual variants or taxonomic problems.

Because of generous spacing and reasonably good quality paper, *Grasses of the Soviet Union* is produced in two volumes each of which is roughly the same size as the complete Russian work. It resembles the translations of *Flora U.R.S.S.* carried out by the Israel Programme for Scientific Translations in that the Russian page-numbers are placed in the margin, and the index entries all refer to them. In the contents pages, however, entries refer to the English-version page-numbers, a quirk to which users will soon become accustomed. The printing is clear and the binding seems to be adequate.

This work is literally essential to any serious grass taxonomist or breeder, and will remain a standard reference work for many decades. Agrostologists the world over are indebted to the publishers for making this authoritative masterpiece available more widely. □

C.A. Stace is Reader in Plant Taxonomy and Head of the Department of Botany at the University of Leicester.

The daily Aztec

Warwick Bray

The Aztecs. By Brian M. Fagan. W.H. Freeman: 1984. Pp.322. Hbk \$27.95, £26.95; pbk \$14.95, £13.95.

IN THE 1950s, when Brian Fagan and I were students together, he was about to become an Africanist and I was still a European prehistorian. Since then, each of us has written a popular book on the Aztecs, his having just been published.

Although separated by 16 years, there is more than a passing resemblance between the two studies, not only in format, but also in the illustrations and in the choice of quotations from sixteenth-century sources. This is some indication of the continuing importance of the rare eyewitness accounts left by those who took part in the conquest of Mexico (Hernando Cortes and Bernal Diaz) and by the missionary friars (Sahagún, Motolinia and Durán) who followed closely behind. Since there are at least two other "daily life" books in circulation, and using the same basic sources, one should not take too much notice of the publisher's claim that the present work is unique. As the most recent of its genre it is, however, the most up to date.

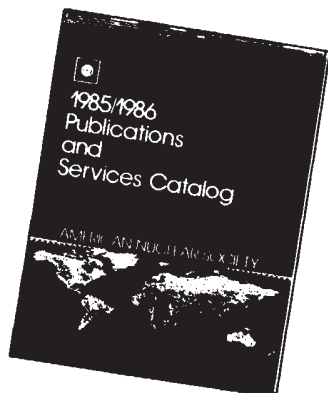
Professor Fagan has established a solid, and well-deserved, reputation as an archaeological popularizer who can be relied on to say the right things in a lively and comprehensible style. He has done so again. Little of what he tells us is new, but, like a good journalist, he has mastered the readily available literature and has checked his facts with the experts. He has also avoided the temptation, which afflicts so many writers on the Aztecs, of overemphasizing the spectacular or the horrific — the gold, temples and human sacrifices — at the expense of the more mundane aspects of daily existence. The end product is a trustworthy and well-balanced introduction to Aztec history and customs, very much in the tradition of descriptive ethnography. Whatever can be seen or can be readily comprehended, whether it is physical (clothing, buildings, farming tools and so on) or behavioural (warfare, marriage, worship) is accurately summarized.

What I found to be missing, though it would perhaps frighten off the non-specialist reader, is *analysis* of the meaning behind the picture: the nature of the Aztec economy, the philosophical ideas behind the poetry and the religious ceremonies, the principles underlying Aztec law and so on. Instead, the book presents an observer's view — what you would see if you were there — and does it very well. This, probably, is as much as most people want. But it does not add up to the "definitive book" promised by the publisher's blurb. □

Warwick Bray is Reader in Latin American Archaeology at the University of London.

Publications & Services Catalog of Nuclear Topics

Available free from the



American Nuclear Society
555 N. Kensington Ave.
La Grange Park, IL 60525
(312) 352-6611

Environmental Sciences

Air Pollution Modeling and Its Application III. C. DE WISPELAERE (ed.). Plenum Press: 1984. Pp. 727. ISBN 0-306-41491-0. Np.

Assessment of Toxic Agents at the Workplace: Roles of Ambient and Biological Monitoring. By A. BERLIN *et al.* Martinus Nijhoff: 1983. Pp. 634. ISBN 0-89838-6. Dfl. 265, \$98.50, £67.25.

Atmosphäre und Umwelt: Chemische Prozesse Menschliche Eingriffe. By PETER FABIAN. Springer-Verlag: 1984. Pp. 115. ISBN 3-540-12863-8. DM 24, \$9.40.

Biological and Environmental Effects of Arsenic. Topics in Environmental Health, Vol. 6. BRUCE A. FOWLER (ed.). Elsevier: 1983. Pp. 281. ISBN 0-444-80513-3. Dfl. 210, \$89.50.

Chemistry and Analysis of Hydrocarbons in the Environment. J. ALBAIGES, R.W. FREI and E. MERIAN (eds). Gordon & Breach: 1983. Pp. 313. ISBN 0-677-06140-4. \$55.

Development and the Environmental Crisis: Red or Green Alternatives? By MICHAEL REDCLIFT. Methuen: 1984. Pp. 149. Hbk ISBN 0-416-32130-5; pbk ISBN 0-416-32140-2. Hbk £9.50.

Environment and Enforcement: Regulation and the Social Definition of Pollution. By KEITH HAWKINS. Oxford University Press: 1984. Pp. 253. Hbk ISBN 0-19-827511-0; pbk ISBN 0-19-827514-5. Hbk £15, pbk £6.95.

Forest Policy: A Contribution to Resource Development. Forestry Sciences. F.C. HUMMEL (ed.). Martinus Nijhoff/Dr W. Junk: 1984. Pp. 310. ISBN 90-247-2883-5. £40, \$60.

Health Issues Related to Metal and Nonmetallic Mining. By WILLIAM L. WAGNER *et al.* Butterworth: 1983. Pp. 517. ISBN 0-250-40610-1. £48.

Liquid Chromatography in Environmental Analysis. JAMES F. LAWRENCE (ed.). Humana Press: 1984. Pp. 392. ISBN 0-89603-045-8. \$55.

Mutagenicity, Carcinogenicity, and Teratogenicity of Industrial Pollutants. MICHELINE KIRSCH-VOLDERS (ed.). Plenum: 1984. Pp. 336. ISBN 0-306-41148-2. Np.

The Urban Atmosphere — Sydney a Case Study. J.N. CARRAS and G.M. JOHNSON (eds). CSIRO: 1983. Pp. 655. Pbk ISBN 0-643-03479-X. \$25.

Victims of the Environment: Loss from Natural Hazards in the United States, 1970-1980. By P.H. ROSSI *et al.* Plenum: 1983. Pp. 238. ISBN 0-306-41413-9. \$24.50.

Wastes in the Ocean. Vol. 3 Radioactive Wastes and the Ocean. P. KILHO PARK *et al.* (eds). Wiley: 1983. Pp. 522. ISBN 0-471-09770-5. £69.95.

Biological Sciences

Advanced Plant Physiology. MALCOLM B. WILKINS (ed.). Pitman: 1984. Pp. 514. ISBN 0-273-01853-1. £17.50.

Algal Symbiosis: A Continuum of Interaction Strategies. LYNDA J. GOFF (ed.). Cambridge University Press: 1983. Pp. 216. ISBN 0-521-25541-4. £25, \$42.50.

Animal Behavior: An Evolutionary Approach, 3rd Edn. By JOHN ALCOCK. Sinauer: 1984. Pp. 596. ISBN 0-87893-021-3. Np.

The Anterior Pituitary Gland. A.S. BHATNAGAR (ed.). Raven: 1983. Pp. 471. ISBN 0-89004-759-6. \$61.

Aquatic Toxicology. Vol. 13. J.O. NRIAGU (ed.). Wiley: 1983. Pp. 525. ISBN 0-471-88901-6. £73.75, \$103.45.

Arteriosclerotic Brain Disease. G. CREPALDI *et al.* (eds). Raven: 1983. Pp. 285. ISBN 0-89004-886-X. \$61.

Assay of Calcium-regulating Hormones. DANIEL D. BIKIE (ed.). Springer-Verlag: 1983. Pp. 271. ISBN 3-540-90841-2/0-387-90841-2. DM 88, \$34.20.

Astrocytes: Normal, Reactive, and Neoplastic. By P.E. DUFFY. Raven: 1983. Pp. 236. ISBN 0-89004-996-3. \$46.

Atherosclerosis Reviews. Vol. 1. A.M. GOTTO Jr and R. PAOLETTI (eds). Raven: 1983. Pp. 264. ISBN 0-89004-910-6. \$58.50.

Biochemical Plant Pathology. J.A. CALLOW (ed.). Wiley: 1983. Pp. 484. ISBN 0-471-90092-3. £34.50, \$65.

Biological Magnetic Resonance, Vol. 5. L.J. BERLINER and J. REUBEN (eds). Plenum: 1983. Pp. 303. ISBN 0-306-41293-4. \$45.

Biology of Animal Behavior. By JAMES W. GRIER. Times Mirror/Mosby: 1984. Pp. 639. ISBN 0-8016-1971-8. £24.

Biostatistical Analysis, 2nd Edn. By JERROLD H. ZAR. Prentice Hall: 1984. Pp. 718. ISBN 0-13-077925-3. Np.

Birds of Southern California's Deep Canyon. By W.W. WEATHERS. University of California Press: 1983. Pp. 266. ISBN 0-520-04754-0. £29.50, \$45.50.

Bone Marrow: Structure and Function. By M. TAVASSOLI and J.M. YOFFEY. Alan R. Liss: 1983. Pp. 312. ISBN 0-8451-0226-5. £37.

The Botanical World. By DAVID K. NORTHINGTON and J.R. GOODIN. Blackwell Scientific: 1984. Pp. 647. ISBN 0-8016-1893-2. £24.

Calcium-Binding Proteins 1983. Developments in Biochemistry, Vol. 25. B. DE BERNARD *et al.* (eds). Elsevier Biomedical: 1983. Pp. 471. ISBN 0-444-80537-0. \$103.75, Dfl. 244.

Calcium in Biology. Vol. 6 in the Metal Ions in Biology Series. THOMAS G. SPIRO (ed.). Wiley: 1983. Pp. 278. ISBN 0-471-88543-6. Np.

Cancer and the Cardiopulmonary System. By M. KHALIL ALI and MICHAEL S. EWER. Raven Press: 1983. Pp. 254. ISBN 0-89004-606-9. \$56.

Cells & Organelles, 3rd Edn. By ERIC HOLTZMAN and ALEX B. NOVIKOFF. Saunders College Publishing: 1984. Pp. 660. Pbk ISBN 0-03-049461-3. Np.

Chemistry and Biology of Pteridines: Pteridines and Folic Acid Derivatives. J.A. BLAIR (ed.). Walter de Gruyter: 1983. Pp. 1070. ISBN 3-11-008560-7. DM 280.

The Chemistry of Enzyme Action. New Comprehensive Biochemistry Vol. 6. M.I. PAGE (ed.). Elsevier: 1984. Pp. 568. ISBN 0444-80504-4. Dfl. 179, \$69.

Clinical Cardiovascular and Pulmonary Physiology. By C. ROSENDORFF. Raven: 1983. Pp. 382. Pbk ISBN 0-89004-919-X. \$22.

Clinical Gastroenterology. R.G. FARMER, E. ACHKAR and B. FLESHLER (eds). Raven: 1983. Pp. 628. ISBN 0-89004-780-4. \$68.50.

Communication and Expression in Hoofed Mammals. By FRITZ R. WALTHER. Indiana University Press: 1984. Pp. 423. ISBN 0-253-31380-5. \$32.50.

Comparative Pathobiology. Vol. 5 Structure of Membranes and Receptors. THOMAS C. CHENG (ed.). Plenum: 1984. Pp. 297. ISBN 0-306-41503-8. \$49.50.

A Complete Checklist of the Birds of the World. By RICHARD HOWARD and ALICK MOORE. Macmillan London: 1984. Pp. 732. Pbk ISBN 0-333-36229-2. Pbk £7.95.

Current Methods in Cellular Neurobiology, Vol. 1: Anatomical Techniques. J.L. BARKER and J.F. McKELVY (eds). Wiley: 1983. Pp. 325. ISBN 0-471-09328-9. £56.50, \$79.

Current Methods in Cellular Neurobiology, Vol. 2: Biochemical Techniques. JEFFREY L. BARKER and JEFFREY F. McKELVY (eds). Wiley: 1984. Pp. 319. ISBN 0-471-09344-0. Np.

Current Topics in Membranes and Transport, Vol. 19: Structure, Mechanism, and Function of the Na/K Pump. F. BRONNER and A. KLEINZELLER (eds). Academic: 1983. Pp. 1,043. ISBN 0-12-153319-0. \$95.

Cutaneous Toxicity. V.A. DRILL and P. LAZAR (eds). Raven: 1983. Pp. 288. ISBN 0-89004-935-5. \$60.

Cytogenetics of the Mammalian X Chromosome, Part A: Basic Mechanisms of X Chromosome Behavior. A.A. SANDBERG (ed.). Alan R. Liss: 1983. Pp. 514. ISBN 0-8451-2402-1. £75.

Cytogenetics of the Mammalian X Chromosome, Part B: X Chromosome Anomalies and Their Clinical Manifestations. A.A. SANDBERG (ed.). Alan R. Liss: 1983. Pp. 514. ISBN 0-8451-2403-X. £75.

The Dawn of Animal Life: A Biohistorical Study. By MARTIN F. GLAESSNER. Cambridge University Press: 1984. Pp. 244. ISBN 0-521-23507-3. £25, \$49.50.

Developing and Regenerating Vertebrate Nervous Systems. Neurology and Neurobiology, Vol. 6. P.W. COATES, R.R. MARKWALD and A.D. KENNY (eds). Alan R. Liss: 1983. Pp. 284. ISBN 0-8451-2705-5. \$56.

Developmental Biology, 2nd Edn. By LEON W. BROWDER. Saunders College Publishing: 1984. Pp. 748. ISBN 0-03-063506-3.

Diseases of Marine Animals. Vol. II Introduction Bivalvia to Scaphopoda. O. KINNE (ed.). Biologische Anstalt Helgoland, Hamburg, FRG: 1983. Pp. 1,038. ISBN 3-9800818-0-X. Np.

Disturbance and Ecosystems: Components of Response. Ecological Studies, Vol. 44. H.A. MOONEY and M. GODRON (eds). Springer-Verlag: 1983. Pp. 292. ISBN 3-540-12454-3/0-387-12454-3. DM 128, \$49.70.

Diving and Marine Biology: The Ecology of the Sublittoral. Cambridge Studies in Modern Biology 3. By GEORGE F. WARNER. Cambridge University Press: 1984. ISBN 0-521-25751-4. Np.

Earthworm Biology. Studies in Biology, No. 161. By J.A. WALLWORK. Edward Arnold: 1983. Pp. 58. Pbk ISBN 0-7131-2884-4. £2.65.

Eating and Its Disorders. Research Publications, Association for Research in Nervous and Mental Disease, Vol. 62. A.J. STUNKARD and E. STELLAR (eds). Raven: 1983. Pp. 302. ISBN 0-89004-891-6. \$56.

Ecology: Principles and Practice. By W.H. DOWDESSELL. Heinemann Educational: 1984. Pp. 312. Pbk ISBN 0-435-60226-8. Pbk £6.95.

Ernährung und Stoffwechsel der Pflanze. By K. MENGEL. Gustav Fischer: 1984. Pp. 431. DM 39.

Ethnopharmacology: Primate Models of Neuropsychiatric Disorders. K.A. MICZEK (ed.). Alan R. Liss: 1983. Pp. 350. ISBN 0-8451-0131-5. \$56.

Eukaryotic Gene Expression. AJIT KUMAR (ed.). Plenum: 1984. Pp. 229. ISBN 0-306-41532-1. \$35.

Evolution of Hormone-Receptor Systems. UCLA Symposia on Molecular and Cellular Biology New Series, Vol. 6. RALPH A. BRADSHAW and GORDON N. GILL (eds). Alan R. Liss: 1983. Pp. 526. ISBN 0-8451-2605-9. £67.

Exploring Biology, 2nd Edn. By PAMELA S. CAMP and KAREN ARMS. Saunders College Publishing: 1984. Pp. 591. ISBN 0-03-063372-9. Np.

The Fauna of the Hortobágy National Park. Vol. 2. S. MAHUNKA (ed.). Akadémiai Kiadó: 1983. Pp. 489. ISBN 963-05-3198-4. £24.50.

Flowering Plants of Wales. By R.G. ELLIS. National Museum of Wales: 1983. Pp. 338. ISBN 0-7200-0271-0. £12.

Formaldehyde: Toxicology, Epidemiology, Mechanisms. By J.J. CLARY, J.E. GIBSON and R.S. WARITZ. Dekker: 1983. Pp. 296. ISBN 0-8247-7025-0. \$45.

Functional Neuroanatomy. N.J. STRAUSFELD (ed.). Springer-Verlag: 1983. Pp. 426. ISBN 3-540-12742-9/0-387-12742-9. DM 198, \$76.90.

Functions of Glutathione: Biochemical, Physiological, Toxicological and Clinical Aspects. A. LARSSON *et al.* (eds). Raven: 1983. Pp. 423. ISBN 0-89004-908-4. \$73.50.

General Zoology, 6th Edn. By CLAUDE A. VILLEE *et al.* Saunders College Publishing: 1984. Pp. 856. ISBN 0-03-062451-7. Np.

Genetics, 2nd Edn. By CHARLOTTE J. AVERS. Willard Grant: 1984. Pp. 644. ISBN 0-87150-779-X. Np.

Globin Gene Expression and Hematopoietic Differentiation. G. STAMATOYANNOPOULOS and A.W. NIENHUIS (eds). Alan R. Liss: 1983. Pp. 560. ISBN 0-8451-0134-X. £91.

Glutamine, Glutamate, and Gaba in the Central Nervous System. Neurology and Neurobiology, Vol. 7. LEIF HERTZ *et al.* (eds). Alan R. Liss: 1984. Pp. 720. ISBN 0-8451-2706-3. £66.

Handbook of Cognitive Neuroscience. MICHAEL S. GAZZANIGA (ed.). Plenum: 1984. Pp. 416. ISBN 0-306-41290-X. Np.

A History of Biochemistry: Selected Topics in the History of Biochemistry. G. SEMENZA (ed.). Elsevier: 1983. Pp. 404. ISBN 0-444-80507-9. \$80.75, Dfl. 210.

Hormones and Behaviour in Higher Vertebrates. J. BALTHAZART, E. PROVE and R. GILLES (eds). Springer-Verlag: 1983. Pp. 489. ISBN 3-540-12576-0/0-387-12576-0. DM 128, \$49.50.

Hormones and Metabolic Control: A Medical Student's Guide to Control of Various Aspects of Normal and Abnormal Metabolism. By DAVID A. WHITE *et al.* Edward Arnold: 1984. Pp. 106. Pbk ISBN 0-7131-4437-8. Pbk £5.95.

Human Genetics. By ELOF AXEL CARLSON. D.C. Heath: 1984. Pp.432. ISBN 0-669-05559-X. Np.

Immune-Deficient Animals. B. SORDAT (ed.). Karger: 1984. Pp.445. ISBN 3-8055-3741-7. SwFr. 195, DM 234, \$117.

Immunology: An Introduction. By IAN R. TIZARD. Saunders: 1984. Pp.428. ISBN 0-03-060277-7. Np.

The Importance of Islets of Langerhans for Modern Endocrinology. 12th Workshop Conference HOECHST, KONRAD F. FEDERLIN and JOSEF SCHOLTHOLT (eds). Raven: 1983. Pp.253. ISBN 0-89004-939-4. \$46.

Integrated Principles of Zoology, 7th Edn. By CLEVELAND P. HICKMAN et al. Times Mirror/Mosby: 1984. Pp.1,065. ISBN 0-8016-2173-9. £27.50.

An Introduction to Epidemiology, 2nd Edn. By M. ALDERSON. Macmillan Press, London: 1984. Pp.335. Hbk ISBN 0-333-35014-6; pbk ISBN 0-333-35015-4. Hbk £20; pbk £8.95.

Laboratory Studies in Integrated Zoology, 6th Edn. By FRANCES M. HICKMAN and CLEVELAND P. HICKMAN Jr. Times Mirror/Mosby: 1984. Pp.465. Pbk ISBN 0-8016-2178-X. Pbk £12.

Lactation: Physiology, Nutrition, and Breast-Feeding. M.C. NEVILLE AND M.R. NEIFERT (eds). Plenum: 1983. Pp.466. ISBN 0-306-41311-6. \$49.50.

Macromolecules: Structure and Properties, 2nd Edn. By HANS-GEORG ELIAS. Plenum: 1984. Pp.521. ISBN 0-306-41007-X. Np.

Maldescensus Testis Operative Andrology 2. Progress in Reproductive Biology and Medicine, Vol.10. A. KELAMI and J.P. PRYOR (eds). Karger: 1984. Pp.176. ISBN 3-8055-3791-3. SwFr. 166, DM 199, \$99.50.

Mast Cell Activation and Mediator Release, Progress in Allergy, Vol.34. KIMISHIGE ISHIZAKA (ed.). Karger: 1984. Pp.336. ISBN 3-8055-3713-1. SFr. 198, DM 237, \$118.75.

Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract. ADRIAN ALLEN et al. (eds). Raven: 1983. Pp.416. ISBN 0-89004-317-5. \$60.

Methods in Biogenic Amine Research. S. PARVEZ et al. (eds). Elsevier Biomedical: 1983. Pp.1,028. ISBN 0-444-80496-X. \$268, Dfl. 630.

Modes and Mechanisms of Microbial Growth Inhibitors. Antibiotics VI. F.E. HAHN (ed.). Springer-Verlag: 1983. Pp.343. ISBN 3-540-12169-2/0-387-12169-2. DM 268, \$104.

Neural Tissue Transplantation Research. ROBERT B. WALLACE and GOPAL P. DAS (eds). Springer-Verlag: 1983. Pp.243. ISBN 3-540-90833-1/0-387-90833-1. DM 108, \$41.90.

Normal and Neoplastic Hematopoiesis, Vol.9: UCLA Symposia on Molecular and Cellular Biology New Series. DAVID W. GOLDE and PAUL A. MARKS (eds). Alan R. Liss: 1983. Pp.594. ISBN 0-8451-2608-3. £73.

Paediatric Oncology. Recent Results in Cancer Research 88. W. DUNCAN (ed.). Springer-Verlag: 1983. Pp.116. ISBN 3-540-12349-0/0-387-12349-0. DM 98, \$38.10.

Parasitic Diseases, Vol.2 The Chemotherapy. JOHN M. MANSFIELD (ed.). Marcel Dekker: 1983. Pp.248. ISBN 0-8247-7050-1. SwFr. 150.

The Pathology of Tropical Food Legumes: Disease Resistance in Crop Improvement. By D.J. ALLEN. Wiley: 1983. Pp.413. ISBN 0-471-10232-6. £33.50.

PCBs: Human and Environmental Hazards. By FRANK M. D'LTRI and MICHAEL A. KAMRIN. Butterworth: 1983. Pp.443. ISBN 0-250-40598-9. £50.

Pharmaceutical Microbiology 3rd Edn. W.B. HUGO & A.D. RUSSELL (eds). Blackwell Scientific: 1983. Pp.470. ISBN 0-623-01048-7. Pbk £14.80.

Pharmacokinetic Basics for Drug Treatment. LESLIE Z. BENET et al. (eds). Raven: 1983. Pp.466. ISBN 0-89004-874-6. \$49.

Plant Breeding Reviews, Vol.1. JULES JANICK (ed.). Croom Helm/AVI Publishing: 1983. Pp.397. ISBN 0-7099-1434-2. Np.

Plant Pests and Their Control, revised edition. By PETER G. FENEMORE. Butterworths: 1984. Pp.280. ISBN 0-407-00304-5. £15.

Polymers in Medicine: Biomedical and Pharmacological Applications. Polymer Science and Technology, Vol.23. E. CHIPELLINI and P. GIUSTI (eds). Plenum: 1983. Pp.420. ISBN 0-306-41360-4. \$57.50.

A Primer of Behavioural Pharmacology. By PETER L. CARLTON. W.H. Freeman: 1983. Pp.301. ISBN 0-7167-1451-5. £11.95.

Principles of Real-Time Sonography in Modern Obstetrics: A Handbook for the Practising Physician. By N. PERONE. Raven: 1984. Pp.173. ISBN 0-89004-999-8. \$42.50.

The Problem-Oriented Medical Record of High-Risk Obstetrics. Plenum: 1984. Pp.486. ISBN 0-306-41325-6. Np.

Applied Biological Sciences

Advances in Biochemical Engineering/Biotechnology: Microbial Activities. A. FIECHTER (ed.). Springer-Verlag: 1983. Pp.200. ISBN 3-540-12791-7/0-387-12791-7. DM 86, \$33.40.

Human Tumor Markers: Biological Basis and Clinical Relevance. H.E. NIEBURGS, G.D. BIRKMEYER and J.V. KLAVINS (eds). Alan R. Liss: 1983. Pp.346. ISBN 0-8451-0228-1. \$56.

Thrombosis and Cardiovascular Diseases. Advances in Experimental Medicine and Biology, Vol.164. ANTONIO STRANO (ed.). Plenum: 1984. Pp.426. ISBN 0-306-41261-6. £59.50.

Urological Complications in Gynecological Surgery and Radiotherapy. J. KUNZ. Karger: 1984. Pp.220. ISBN 3-8055-3759-X. DM 162, \$81.

Vascular Perfusion in Cancer Therapy. K. SCHWEMMLE and K. AIGNER (eds). Springer-Verlag: 1983. Pp.295. ISBN 3-540-12346-6/0-387-12346-6. DM 128, \$49.70.

Vertebrobasilar Arterial Occlusive Disease: Medical and Surgical Management. RAMON BERGUER and RAYMOND BAUER (eds). Raven: 1984. Pp.336. ISBN 0-89004-984-X. \$56.

Viral Hepatitis: Second International Max Von Pettenkofer Symposium. Infectious Diseases and Antimicrobial Agents Series, Vol.4. L.R. OVERBY, F. DEINHARDT and J. DEINHARDT (eds). Dekker: 1983. Pp.336. ISBN 0-8247-7046-3. SwFr. 122.

Psychology

Adolescence: A Social Psychological Analysis, 3rd Edn. By HANS SEBALD. Prentice Hall: 1984. Pp.336. ISBN 0-13-008516-2. £22.75.

Annals of Theoretical Psychology, Vol.1 JOSEPH R. ROYCE and LEENDERT P. MOS (eds). Plenum: 1984. Pp.325. ISBN 0-306-41327-2. Np.

Childhood Cancer: Impact on the Family. The Downstate Series of Research in Psychiatry and Psychology, Vol.5. ADOLPH E. CHRIST and KALMAN FLOMENHAFT (eds). Plenum: 1984. Pp.302. ISBN 0-306-41495-3. \$45.

Child Psychiatry: A Plan for the Coming Decades. IRVING PHILIPS et al. (eds). American Academy of Child Psychiatry: 1983. Pp.115. Pbk ISBN 0-83-7146. Np.

Clinical Perspectives on the Supervision of Psychoanalysis and Psychotherapy. LEPOLD CALIGOR et al. (eds). Plenum: 1984. Pp.281. ISBN 0-306-41403-1. Np.

A Critical Psychology: Interpretation of the Personal World. By EDMUND V. SULLIVAN. Plenum: 1984. Pp.195. ISBN 0-306-41434-1. Np.

Drugs for Mental Illness: A Revolution in Psychiatry. By M.E. LICKY and B. GORDON. W.H. Freeman: 1983. Pp.349. Hbk ISBN 0-7167-1457-4; pbk ISBN 0-7167-1458-2. Hbk np; pbk £9.95.

Introduction to Psychology: An Integrated Approach. By PETER LLOYD et al. Fontana: 1984. Pp.815. Pbk ISBN 0-00-636267-2. Pbk £5.95.

Issues in Psychotherapy Research. MICHEL HERSEN et al. (eds). Plenum: 1984. Pp.423. ISBN 0-306-41401-5. Np.

Modern Eclectic Therapy: A Functional Orientation to Counseling and Psychotherapy. By J. HART. Plenum: 1983. Pp.394. ISBN 0-306-41213-6. \$37.50.

The Origins of Depression: Current Concepts and Approaches. Dahlem Workshop Reports, Vol.26. J. ANGST (ed.). Springer-Verlag: 1983. Pp.471. ISBN 3-540-12451-9/0-387-12451-9. DM 58, \$19.50.

Psychological Perspectives of Essential Hypertension: Etiology, Maintenance and Treatment. By WOLFGANG LINDEN. Karger: 1984. Pp.130. ISBN 3-8055-3662-3. SwFr. 69, DM 83, \$41.50.

Psychology, 2nd Edn. JOHN M. DARLEY et al. (eds). Harvey University Press: 1984. Pp.676. ISBN 0-13-733147-9. £25.60.

Psychoneuroendocrine Dysfunction. NAND-KUMAR S. SHAH and ALEXANDER G. DONALD (eds). Plenum: 1984. Pp.636. ISBN 0-306-41320-5. Np.

Psychosocial Constructs of Alcoholism and Substance Abuse. B. STIMMEL (ed.). Haworth Press: 1983. Pp.117. ISBN 0-86656-244-3. \$14.95.

Sex Roles and Psychopathology. CATHY SPATZ WIDOM (ed.). Plenum: 1984. ISBN 0-306-41406-6. \$45.

Talks to Teachers on Psychology and to Students on Some of Life's Ideals. The Works of William James. FREDERICK BURKHARDT (ed.). Harvard University Press: 1983. Pp.334. ISBN 0-674-86785-8. Np.

General

Adaptive Control of Ill-Defined Systems. NATO Conference Series II: Systems Science. OLIVER G. SELFRIDGE et al. (eds). Plenum: 1984. Pp.349. ISBN 0-306-41475-9. \$47.50.

Agriculture in China's Modern Economic Development. By N.B. LARDY. Cambridge University Press: 1984. Pp.285. ISBN 0-521-25246-6. £22.50, \$37.50.

The American Darters. By ROBERT A. KUEHNE and ROGER W. BARBOUR. University of Kentucky Press: 1983. Pp.177. ISBN 0-8131-1452-7. £38.25.

Arsenal: Understanding Weapons in the Nuclear Age. By K. TSIPIS. Simon & Schuster: 1984. Pp.342. ISBN 0-671-44073-X. \$16.95.

Biobusiness World Data Base. Draft Report by a U.S. Government Interagency Working Group on Competitive and Transfer Aspects of Biotechnology. Elsevier: 1983. ISBN 0-444-99629-X. Np.

Catastrophe Theory. By V.I. ARNOLD. Springer-Verlag: 1984. Pp.79. Pbk ISBN 3-540-12859-X/0-387-12859-X. DM 16.80, \$6.60.

The Smiling Spleen: Paracelsianism in Storm and Stress. By WALTER PAGE. Karger: 1984. Pp.213. ISBN 3-8055-3707-7. SwFr. 153, DM183, \$91.75.

State of the World 1984: A Worldwatch Institute Report on Progress Toward a Sustainable Society. LESTER R. BROWN et al. (eds). W.W. Norton: 1984. Hbk ISBN 0-393-01835-0; pbk ISBN 0-393-30176-1. Np.

Under the Magnifying Glass. By ALTHEA. Dinosaur Publications: 1984. Pp.24. Hbk ISBN 0-85122-416-4; pbk ISBN 0-85122-415-6. Hbk £2.95, pbk £1.10.

Urban Innovation Abroad: Problem Cities in Search of Solutions. THOMAS L. BLAIR (ed.). Plenum Press: 1984. Pp.414. ISBN 0-306-41492-9. Np.

Will My Baby Be Normal? Everything You Need to Know About Pregnancy. By JONATHAN SCHER and CAROL DIX. Allen Lane: 1984. Pp.240. ISBN 0-7139-1552-8. £7.95.

The Youngest Science: Notes of a Medicine-Watcher. By LEWIS THOMAS. Oxford University Press: 1984. Pp.276. ISBN 0-19-217735-4. £12.50.

History of Science

The Mathematicians' Apprenticeship: Science, Universities and Society in England, 1560-1640. By MORDECHAI FEINGOLD. Cambridge University Press: 1984. ISBN 0-521-25133-8. £22.50, \$37.50.

A Realist Philosophy of Science. By JERROLD L. ARONSON. Macmillan Press: 1984. Pp.278. ISBN 0-333-35329-3. £25.

Reason and the Search for Knowledge: Investigations in the Philosophy of Science. Boston Studies in the Philosophy of Science, Vol.78. By DUDLEY SHAPERE. D. Reidel Publishing: 1984. Pp.438. ISBN 90-277-1551-3. Dfl 155, \$59.50.

Science Under Scrutiny: The Place of History and Philosophy of Science. R.W. HOME (ed.). D. Reidel Publishing: 1983. Pp.182. ISBN 90-277-1602-1. Dfl 90, \$36.

Nucleonics

The following pages include items of interest for workers in the field of nucleonics

● The formation of the Nuclear Counting Exchange, an organization for users of nuclear instrumentation, has been announced by Beckman Instruments, Inc.'s Nuclear Systems Operations. The Nuclear Counting Exchange is designed to promote nuclear counting in research and industrial environments. Users of Beckman liquid scintillation and gamma counters are invited to join. Investigators in the group will share their expertise in solving practical application problems, obtain current information on interfacing and software programs for personal computers in nuclear counting, become aware of new developments in technology, communicate their ideas for future technology, and obtain educational resources not available elsewhere. The Nuclear Counting Exchange will provide a quarterly newsletter, workshops and technical literature.
Circle No. 100 on Reader Service Card.

● Kevex have introduced an 0700 laboratory X-ray fluorescence system. Capable of accepting up to 16 samples, the 0700 system provides a rapid quantitative method of non-destructive multi-element analysis and offers the choice of direct, filtered direct and secondary excitation. To speed up throughput, all user accessible functions are under microprocessor control. These include: sample position; direct or secondary target X-ray tube position; X-ray high voltage; X-ray tube current and transmission filter/collimator selection. The multiple secondary target facility, coupled with the rapidly accessible direct and filtered direct excitation modes, allows sample excitation radiation to be tailored to analysis requirements; background counts can be reduced and relative peak intensities increased.
Circle No. 101 on Reader Service Card.

● Coherent, Inc. of Palo Alto, California, have announced the addition of a power meter to their existing product line. The Series 2000 Power and Energy Meter features a liquid crystal display for digital operation with a unique trend bar (US patent pending) for tuning continuous wave lasers. Meter readout power range is 0 to 2kW depending on head selection with a choice of five models. The meter can also measure pulsed laser energy modes. Other features include zero reset; excess power, over temperature and low battery warning; battery and AC adapter operation; adjustable viewing angle; and analogue or digital outputs for electronic interfacing.
Circle No. 102 on Reader Service Card.



Kevex 0700 X-ray fluorescence system

● VG-354 thermal ionization mass spectrometer is a 90° magnetic sector, single focusing, thermal ionization instrument, incorporating the latest advances in ion optics, source design, multiple ion collection and vacuum expertise. The versatile multi-collector facility, developed by VG Isotopes, allows multi-collection measurement on any isotope group. The system consists of up to eight Faraday cups and a Daly scintillation detector. Four of the collectors are adjustable, either manually or under computer control from outside the vacuum. Short analysis times are possible due to a high proportion of the total available ion beam being collected during a given time period, while the effects of beam fluctuation with respect to time are reduced. Up to 16 samples may be analysed sequentially under computer control, with all data reduction being carried out automatically.
Circle No. 103 on Reader Service Card.

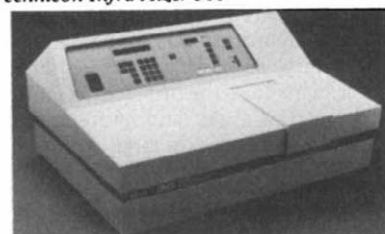
Circle No. 103 on Reader Service Card.

● Now available from Philips is a new material PX-1 which when used to replace the conventional TIAP crystal significantly improves the light performance of the Philips PW 1400 sequential X-ray spectrometer. Known as a multilayer, PX-1 is made using semiconductor manufacturing techniques to deposit alternating layers of heavy and light element atoms on a silicon substrate. The carefully controlled layers, measuring just nanometres in thickness, form a crystal-like repeating structure that acts as an X-ray monochromator. This gives a more than three-fold intensity increase when measuring fluorine, sodium and magnesium.
Circle No. 104 on Reader Service Card.

Circle No. 104 on Reader Service Card.



Technicon Infra Alzer 500



Spectrophotometer DMS 70 from Varian

● Varian has introduced a DMS 70 spectrophotometer to complement the entire Varian series of UV/VIS instruments. The optics with the holographic grating and double beam configuration give efficient performance and the wide wavelength range, from 800 to 200 nm, make the instrument ideal for almost all applications. The use of all reflective optics in a sealed compartment guarantee high performance of the DMS 70. The keyboard control is easy to use, and readout is in absorbance, transmittance or concentration mode. The instrument has a wide range of accessories, featuring a programmable cell changer for the scientist involved in kinetic studies. The sipper and batch sampler are ideal when many samples need to be read at a fixed wavelength: up to 60 samples can be run automatically. A gel scanner, fluorescence and reflectance accessories and a variety of unusual sample handling devices are also available.
Circle No. 105 on Reader Service Card.

Circle No. 105 on Reader Service Card.

● Technicon InfraAlyzer 500 system can be used to investigate and implement the use of the near infrared reflectance technology which offers rapid and precise multi-component analysis allowing significant improvements in analytical productivity and improved process monitoring. New models capable of analysing both liquid and solid samples, and the availability of a wide range of software options, have resulted in an increased recognition of the potential application of the technique to many areas of the chemical industry. The system is already used for rapid routine analysis of food stuffs and agricultural commodities.
Circle No. 106 on Reader Service Card.

Circle No. 106 on Reader Service Card.

● The Lambda 9 is the latest addition to Perkin-Elmer's Lambda series of UV/VIS spectrophotometers. It is a double monochromator, double beam ratio recording instrument, capable of scanning the range from 185nm up to 3200nm in the near IR. This extended wavelength range, together with excellent optical performance, enables the Lambda 9 to be used for a wide range of applications. The optical design of the Lambda 9 provides high energy throughput and accurate focusing of the beam onto the sample to give an excellent signal-to-noise ratio throughout the entire wavelength range of the instrument. Spectral bandwidth can be varied in 0.01nm increments using microcomputer-controlled synchronized slit mechanisms, with a maximum resolution of 0.05nm in the UV/VIS range and up to 20nm band-pass in the NIR. In addition to the standard sample compartment, the optical unit includes a large secondary sample compartment which allows the primary detector to be removed as a unit and an internal integrating sphere or any large custom accessory to be easily installed. The Lambda 9 has an optional RS232C interface for connection to a laboratory computer for further evaluation and storage of spectral data and can be equipped with the many sample handling accessories.

Circle No. 107 on Reader Service Card.

● Recent developments at the Laser Analytics Division of Spectra-Physics, have resulted in a cleaved-coupled-cavity Pb-salt diode laser that operates single mode over limited current and temperature ranges. The new C³ lead-salt laser provides a much greater degree of mode control than has been possible with single-cavity lasers. The new device consists of two coupled cavity sections: the front section performs as an emitting laser and the rear section as a modulator. By varying the modulator current, it is possible to tune the emission from the front section thereby obtaining additional precise control of the laser emission characteristics. The single mode tuning rate — which is sometimes large for mesa-stripped lasers — is considerably reduced when modular current is used as the fine-tuning instrument, a technique which provides improvements in device performance for applications requiring very stable, long term single mode operations.

Circle No. 108 on Reader Service Card.

ADVERTISEMENT

PHORBOL ESTERS

99 + % PURE
PMA (TPA)

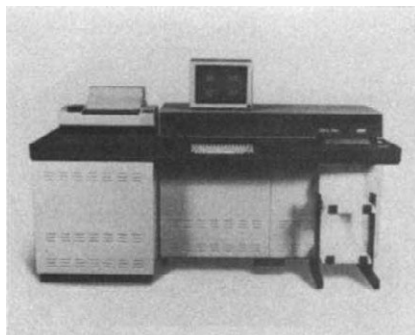
PHORBOL 12-MYRISTATE
13-ACETATE

Cat. #P1880 5mg/\$28⁰⁰

LC SERVICES CORPORATION

165 New Boston Street
Woburn, MA 01801
(617) 938-1700

Circle No. 19 on Reader Service Card.



Bruker MSL pulsed NMR spectrometer

● A new ion chromatography system, the IonChem, has been launched in the UK by Severn Analytical of Gloucester; the system is based on a new conductivity detector which uses a very fast rate bi-polar pulse technique to improve signal-to-noise ratio and thereby allows high-sensitivity detection, without the need for complex post-column suppression systems. The new detector means that measurement temperatures in the cell can be controlled to within $\pm 0.005^\circ\text{C}$. The instrument has a "pulse-free" isocratic solvent delivery system, an insulated column compartment, and also uses the latest polymer-based, high-speed, high-resolution columns. By using a conventional HPLC pump, other detectors can be added to the system, giving greater flexibility than a suppressed system for other chromatographic work.

Circle No. 109 on Reader Service Card.

● New from Burleigh Instruments is their TL Etalon Series offering opportunities for rapid, precise measurement of the mode output of semiconductor lasers. These etalons can be configured to monitor the spectral quality of fibre output from fibres directly coupled to semiconductor lasers, and allow optimization of the fibre coupling to produce near single-mode output from potentially multi-mode semiconductor lasers. Laser fibre packages can also be graded with respect to spectral purity, especially the percentage of single-mode output. These TL Etalons are available in standard configurations from Burleigh Instruments. Also available are wavemeters and high resolution confocal etalons for precision wavelength measurement and frequency stability measurements.

Circle No. 110 on Reader Service Card.

● Amersham Corporation has announced new, commercially available tritium standards which provide a convenient alternative for the rapid quantification of tritium in tissue sections. The product is a multilayer block of polymer designed to be cut by a microtome to give strips of multi-level activities for use as autoradiographic standards. Each block consists of 8 layers of tritiated polymer, arranged in order of increasing specific activity and separated by inactive layers.

Circle No. 111 on Reader Service Card.

● A new spectrometer that covers the range of linewidths from 0.1 Hz to 1 MHz, combining high resolution NMR spectroscopy with the power and versatility of a solids instrument, has been announced by Bruker Instruments, Inc., of Billerica, Massachusetts, USA. Experiments handled by the new MSL unit include: 2D NMR in liquids and solids, variable temperature MAS, wide-line FT, multipulse line narrowing (MREV-8, BR-24, etc), ADRF/ARRF, multiple quantum NMR, NMR diffusion, NMR imaging, in vivo spectroscopy, NQR, and ferromagnetic resonance studies. The MSL system offers: full automation and complete keyboard control, sample changer, colour raster, compu-shim and auto-lock, a new fast and versatile pulse programmer, and provision for interfacing many user devices.

Circle No. 112 on Reader Service Card.

● Philips new automated X-ray powder diffractometer, the PW 1840 compact X-ray diffraction analysis system was first shown at the Pittsburgh Conference in Atlantic City, USA, in March. The new diffractometer at the heart of the system was designed particularly for routine sample screening in industry and health care and permits the fast evaluation of large batches of samples. The new diffractometer incorporates a specially developed solid-state detector and a self-adjusting divergence slit. Integrated with the X-ray source, this unit permits rapid alignment and ensures reproducible operations. Automated operation is provided by a microprocessor control module which enables measurement parameters to be entered by means of function keys. Results may be produced as diffractograms on a chart recorder or as integrated intensities read from the LED display on the control panel. This gives the operator the ability to obtain semi-quantitative analyses rapidly, by selecting a feature of interest on a diffractogram, setting the appropriate scan range and then simply comparing the measured intensities with those obtained from standard samples.

Circle No. 113 on Reader Service Card.

● Information submitted by 85 users of Radiometer's ION85 ion analyzer has been collected in a report titled *The First 85 ION85 Installation*. The report details each installation with respect to ions of interest, sample type, measuring method, and number of daily measurements. The information is listed according to different user groups: agriculture, beverages and food, environmental, pharmaceutical labs, etc. The 20-page booklet covers users in 14 countries.

Circle No. 114 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.